TOTAL SYNTHESIS

OF

(S)-4-HYDROXY-α-LAPACHONE

BY

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ABSTRACT

(S)-4-Hydroxy-α-lapachone has been prepared for the first time. The commercially available compound 2-acetyl-1-naphthol was used as the starting material. The synthesis involved methylation, followed by Baeyer-Villiger oxidation, and hydrolysis of the acetate to give 1-methoxy-2-naphthol. After protecting of the hydroxyl group, t-BuLi was used to form 3-(3',3'-dimethyl-acryloyl)-1-methoxy-2-(methoxymethoxy)-naphthalene. Cyclization and oxidation then gave 4-keto-α-lapachone. Finally enzymic biotransformation by *Mortierella isabellina ATCC 42613* was used to yield the target compound. The enantiomeric excess of the product was determined to be ≥98% by using ^{1}H NMR chiral shift analysis. The overall yield is 8%. The biological activity of *(S)*-4-hydroxy-α-lapachone and its acetate are under investigation.

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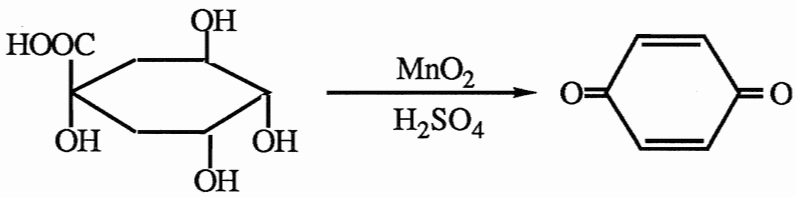
INTRODUCTION

I QUINONES:

1. Historical background

The generic name quinone is derived from the compound discovered in Liebig's laboratory as a product of the oxidation of quinic acid with manganese dioxide and sulfuric acid (Equation 1).[1] Quinic acid, a constituent of cinchona bark and of the coffee bean, is 1,3,4,5-tetrahydroxy-hexahydrobenzoic acid, and has the configuration shown. Its conversion into quinone involves dehydration, decarboxylation, and oxidation. The yellow product was called quinone, or p-benzoquinone; the isomeric o-benzoquinone is known, but m-quinones do not exist.

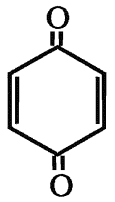
Equation-1:



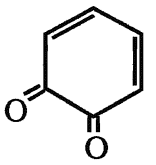
Quinic acid p-Benzoquinone

Quinones are cyclohexadiendiones, but they are named as derivatives of aromatic systems: benzoquinones are derived from benzene, toluquinones from toluene, naphthoquinones from naphthalene, and so on. 'Quinone' is used both as a generic term and as a common name for p-benzoquinone.

One characteristic of quinones is color, and a usual differentiation between *para* and *ortho* quinones is that most of the former are yellow and the majority of the latter are orange or red.



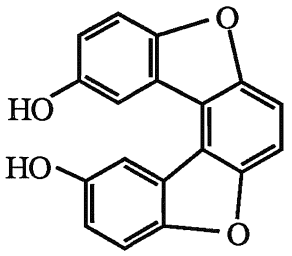
p–benzoquinone
Yellow



o-benzoquinone
Red

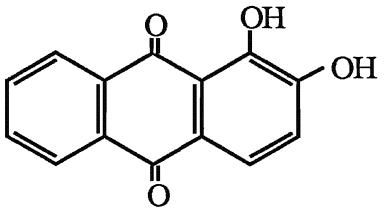
Quinone (p-benzoquinone) is also the end product of oxidation of aniline in sulfuric acid solution with manganese dioxide.[2]

Quinone in the solid state has considerable vapor pressure, and when heated gently sublimes readily to form large yellow crystals. The substance has a characteristic pungent odor and causes sneezing. Quinone readily combines with proteins, probably by Michael addition reactions involving free amino and sulfhydryl groups; it stains the skin and can be used for tanning leather. Quinone is polymerized by acid to a mixture of products, one of which has been identified as the trimer **1** with an o-terphenyl skeleton.[3]



1

Ancient Egyptians and Romans dyed cotton a beautiful red color with a substance that was present in madder root and later identified as an alizarin **2**.[4]



1,2-Dihydroxyanthraquinone(Red)
(Alizarin)

2

In 1868, alizarin was synthesized in the laboratory and this was the first synthesis of a naturally occurring quinone.

By 1950, about 60 quinones from natural sources had been obtained synthetically, at that time about 150 naturally occurring quinones were known. The number of known natural quinones has now

increased to more than 1600, and these quinones are present in all forms of life. Most exhibit some sort of biological activity, notably the bioquinones which play an important role in electron transport in animals and plants.

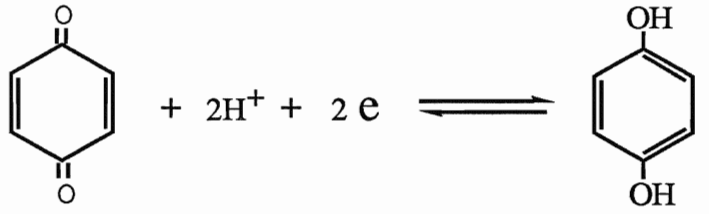
2. Reaction

A. Oxidation and reduction:

Benzoquinones undergo ring fission with oxidizing agents and they may be formed at an intermediate stage in the oxidation of benzene to muconic and maleic acids. It should be noted, however, the biochemical oxidation of benzene leads apparently to the *trans-trans* muconic acid,[5] whereas peracetic acid oxidation of o-quinone affords the *cis-cis* compound.[6]

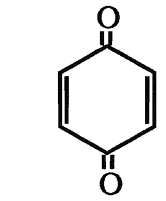
Oxidation-Reduction potentials:

Quinones are cyclic unsaturated diketones so constituted that addition of hydrogen to the terminal oxygen atoms produces dihydroxy derivatives, hydroquinones, that are fully aromatized (Equation-2).

Equation-2: 

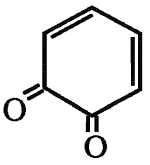
This process is reversible, permitting measurement of the reduction potential, which is an index of the tendency of the quinone to revert to an aromatic molecule. The aromatization process liberates less energy if one or both of the ethylenic bonds are already part of an aromatic nucleus. In other words, the stability of a quinone is increased (potential decreased) when the unsaturation is decreased. The reduction of quinone to hydroquinone in aqueous solution is a rapid, quantitative, and reversible process comparable to the reduction of ferric to ferrous ions, and can be formulated as an electrochemical reaction.

The quantity E_0 is a normal potential characteristic of a specific quinone-hydroquinone system, and is defined as the potential of the half-cell when the hydrogen-ion concentration is unity and the concentration of the quinone, or oxidant, is equal to that of the hydroquinone, or reductant. The following quinones illustrate this effect (Scheme-1); the numbers are the reduction potentials measured at 25^0C.[7]



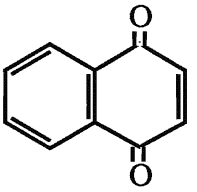
p–Benzoquinone

0.715



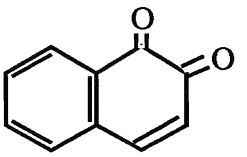
o-Benzoquinone

0.794



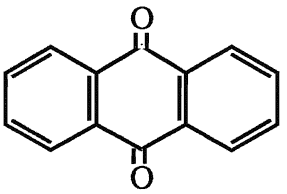
1,4-Naphthoquinone

0.484



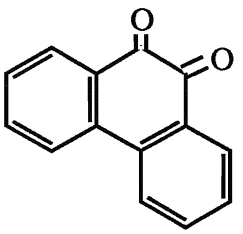
1,2-Naphthoquinone

0.576



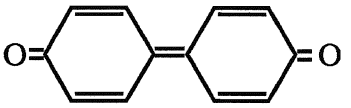
Anthraquinone

0.154



Phenanthraquinone

0.460



Diphenoquinone

0.954

Scheme-1

Diphenoquinone, with the unsaturated conjugated quinonoid system extending throughout two rings, has a very high potential and is a powerful oxidizing agent. In anthraquinone both the otherwise reactive quinonoid double bonds participate in a benzenoid ring system; hence this quinone has a low potential. Conversely, its reduction product, anthrahydroquinone, is a powerful reducing agent.

The influence of a substituent on the reduction potential of a quinone may be predicted qualitatively by consideration of its electron-attracting power. Substituents that donate electrons, i.e., o, p-directing groups (excepting the halogens), lessen the receptivity of the quinone to external electrons, making the formation of the hydroquinone less easy. As would be expected, m-directing substituents such as CN, CO_2H, NO_2, and COR have the opposite effect, making the corresponding hydroquinone less easy to

oxidize. Table 1 shows effect of 2-substituents on the reduction potential of 1,4-naphthoquinone:[8]

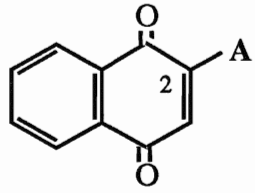
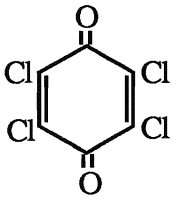


Table: 1

SUBSTITUENT	EFFECT,IN MV.	SUBSTITUENT	EFFECT,IN MV.
$NHCH_3$	-252	$NHCOCH_3$	-67
NH_2	-210	C_6H_5	-32
$N(CH_3)_2$	-181	$OCOCH_3$	-9
OH	-128	Cl	+24
OCH_3	-131	SO_3Na	+69
CH_3	-76	$SO_2C_6H_4CH_3$	+121

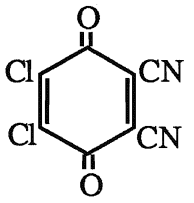
Chloranil and dichlorodicyanobenzoquinone (DDQ) are good oxidation reagents that can convert an alkyl-alkene into a

conjugated diene, a diene into a triene, an alkylbenzene into a styrene derivative (Scheme-2), and so on:



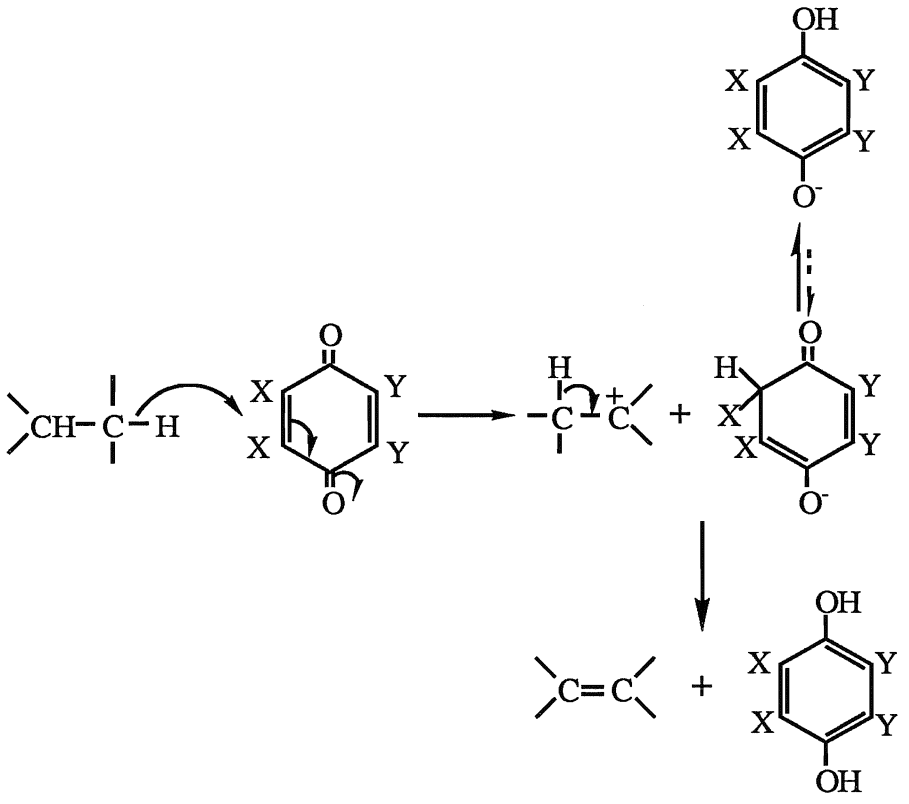
Chloranil

3



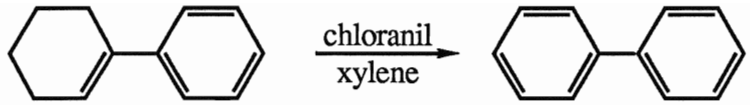
Dichlorodicyanobenzoquinone
DDQ

4

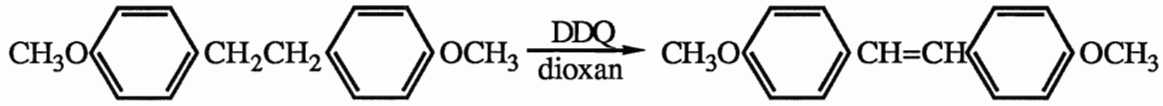


Scheme-2

For example,



(52%)[9]

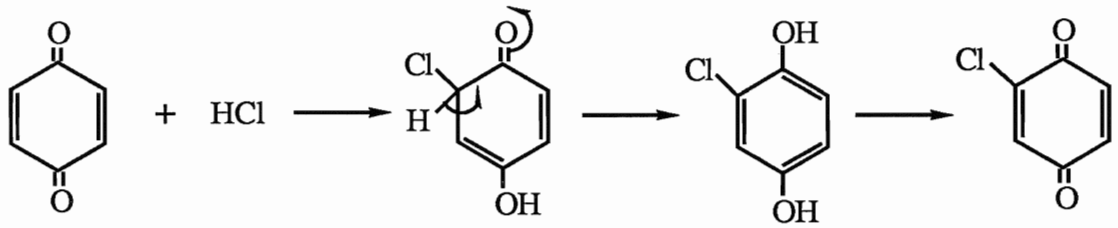


(83%)[9]

B. 1,4-Addition

Addition reactions of quinones involve reduction, and generally a substituted dihydroxybenzene is formed, which may be isolated as such or may undergo oxidation to a substituted quinone at the expense of the unreacted starting material. For example (Equation-3):

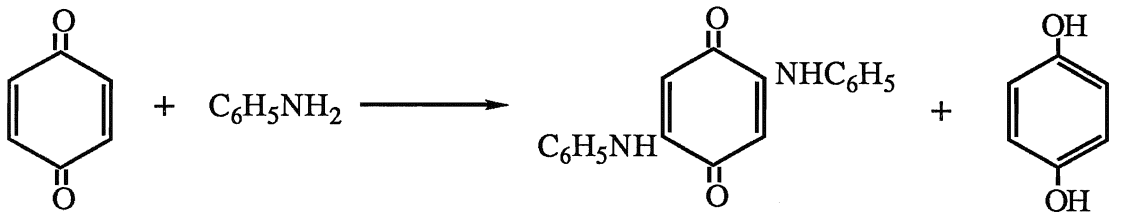
Equation-3:



The first 1,4-addition to quinone was in an experiment by Hofmann in 1863 in which he heated quinone with aniline in alcohol

and obtained 2,5-dianilino-1,4-benzoquinone and hydroquinone.[8] The experiment illustrates the usual orientation in additions: the initially formed anilinoquinone reacts further to give the 2,5-dianilinohydroquinone (Equation-4), which can be oxidized to quinone by starting compound (benzoquinone).

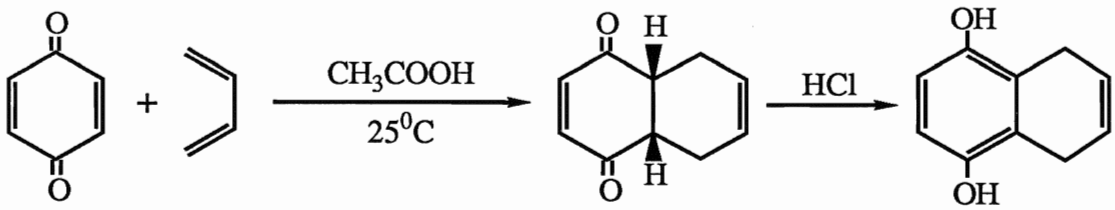
Equation-4:



C. Diels-Alder reaction:

Quinones also have a function as potent dienophiles in the Diels-Alder reaction. An example is the reaction of 1,4-benzoquinone with butadiene, which occurs in acetic acid solution at room temperature. The product may be isolated or it may be treated with HCl, whereupon rearrangement to the more stable hydroquinone form occurs (Equation-5).

Equation-5:

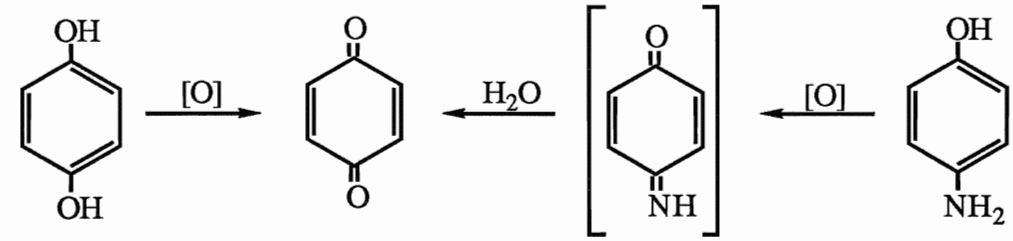


(88%)[9]

3. Preparation

Oxidation is by far the most important reaction for the preparation of quinones. A phenol or an amine is usually the starting material followed by introduction of either a hydroxyl or an amino group in an *ortho* or *para* position and oxidation of the intermediate in acid solution. The initial oxidation product of aminophenol, quinonimine, is extremely sensitive and undergoes hydrolysis to quinone (Equation-6).

Equation-6:



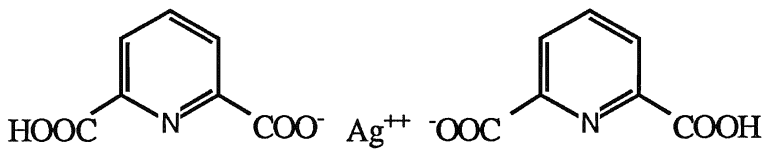
A free radical mechanism has been proposed for these type reactions by Pummerer[11] and Wieland.[12] The usual oxidizing agents

have been used: sodium or potassium dichromate in dilute sulphuric acid, manganese or lead dioxide, ferric chloride or sulphate, and occasionally nitric acid, hydrogen peroxide, bromine or silver oxide. The choice of the oxidizing agent and the conditions is very important not only to the yields, but also for isolation of intermediate products. *Ortho* and *para* diols are easily oxidized to *ortho*- and *para*-quinones. Dipotassium nitrosodisulfonate (Fremy's salt, $(KSO_3)_2NO$) is the most effective reagent for a ring with one OH or NH_2 group (Equation-7).

Equation 7:

$$C_6H_5OH + 2(KSO_3)_2NO \longrightarrow O{=}C_6H_4{=}O + (KSO_3)_2NOH + (KSO_3)_2NH$$

In many cases, hydroquinone dimethyl ethers can be oxidized to their corresponding quinones by using a variety of oxidizing agents,[13] such as nitric acid[13] or argentic oxide.[14] Particularly silver(II) dipicolinate ($(DPAH)_2Ag\cdot H_2O$)[15] **5** and ceric ammonium nitrate (CAN)[16] give of high yields (up to 78%) under mild conditions. There are no reports of convenient methods for conversion of mono-methyl ethers to quinone.

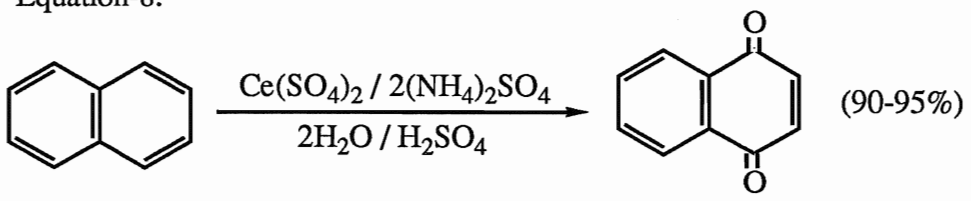


Silver(II) dipicolinate

5

Simple polycyclic aromatic hydrocarbons can be directly oxidized to quinones by various oxidizing agents, but yields are not generally high. There is, however, one report of a high yield for the oxidation of polycyclic aromatic hydrocarbons to quinones with ceric ammonium sulfate (Equation-8).[17,18]

Equation-8:

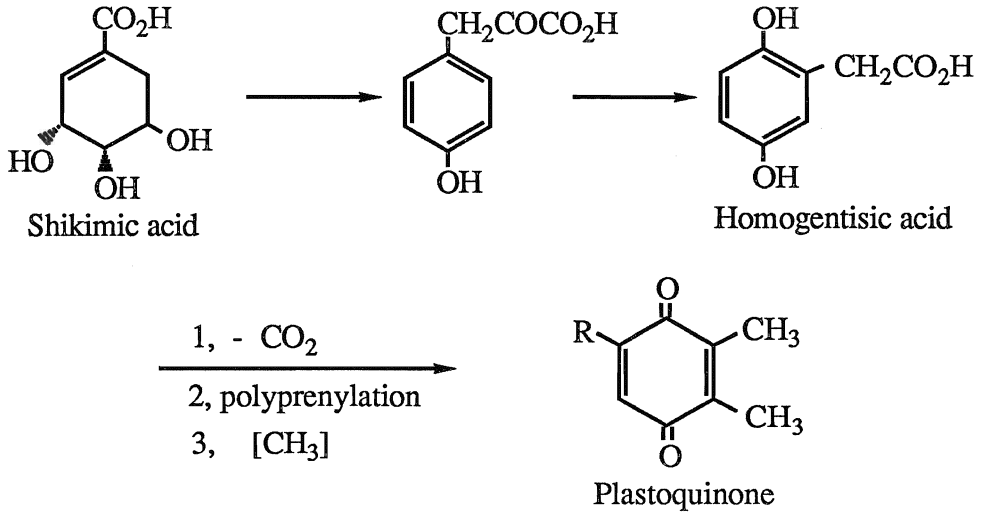


Benzene cannot be oxidized by strong oxidizing agents, but it can be electrolytically oxidized to benzoquinone.[19]

4. Biosynthesis of quinones

It is difficult to systematize the biosynthesis of quinones because it shows such a diversified picture.[20] Benzoquinones can originate either from shikimic acid (Scheme-3), polyketides (Scheme-4), or the mevalonic acid pathway.

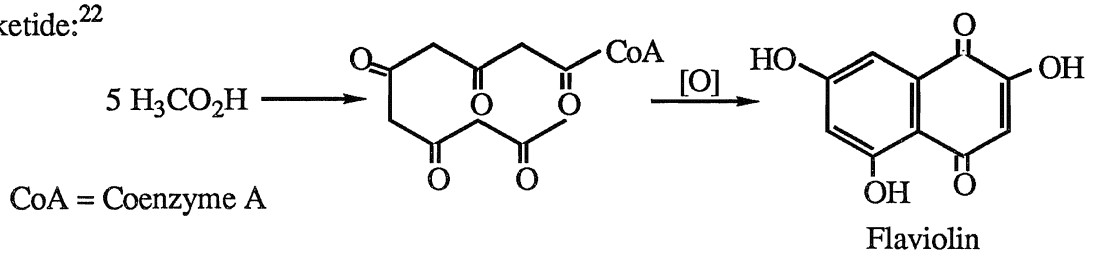
Shikimic acid:[21]



Scheme-3

Naphthoquinones may either by completely synthesized from acetate, e.g. in flaviolin (Schem-4), or they originate from mixed biosynthesis (Scheme-5), e.g. lawsone and vitamin K.

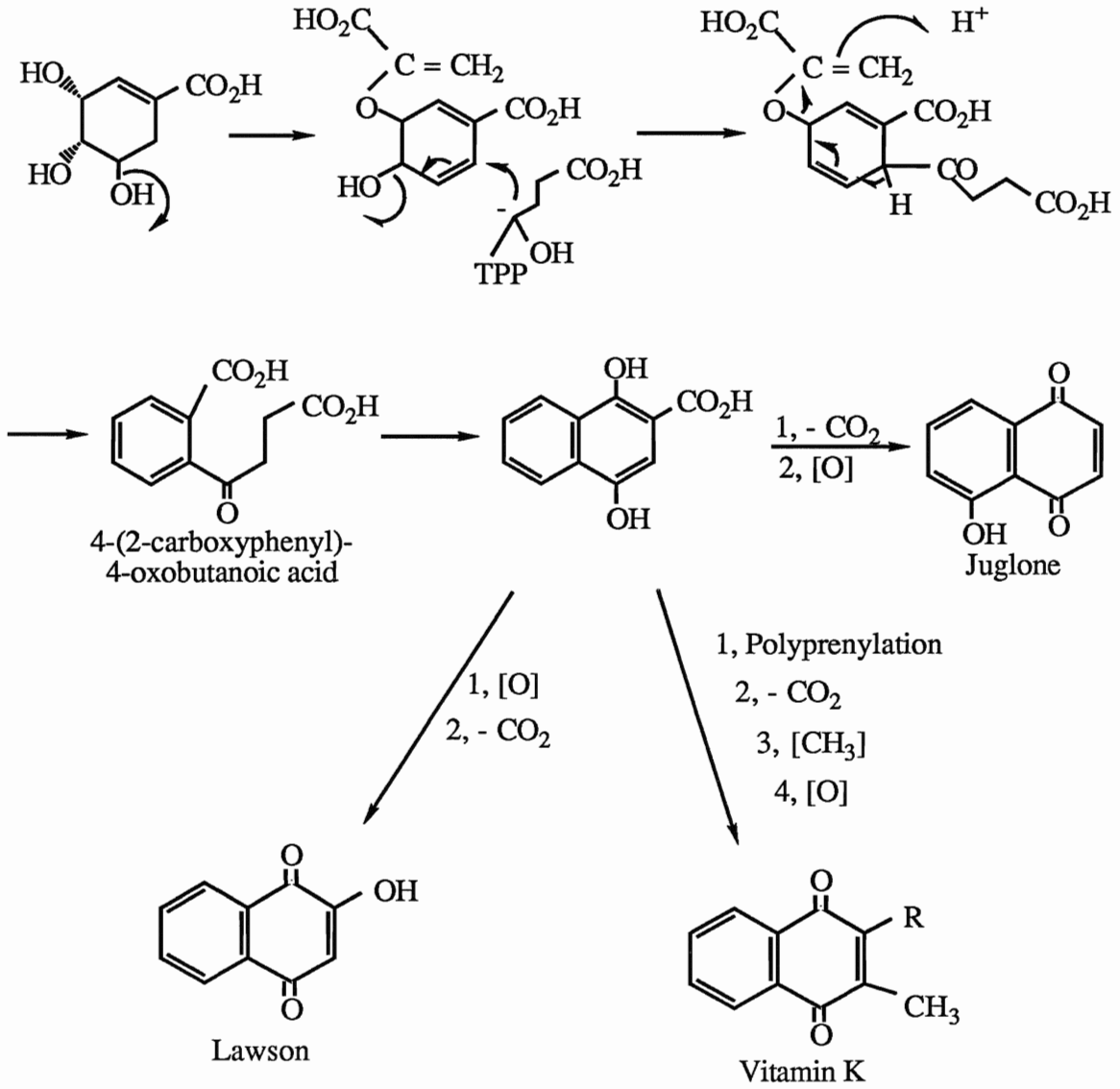
Polyketide:[22]



CoA = Coenzyme A

Scheme-4

Mixed biosynthesis:[23,24]

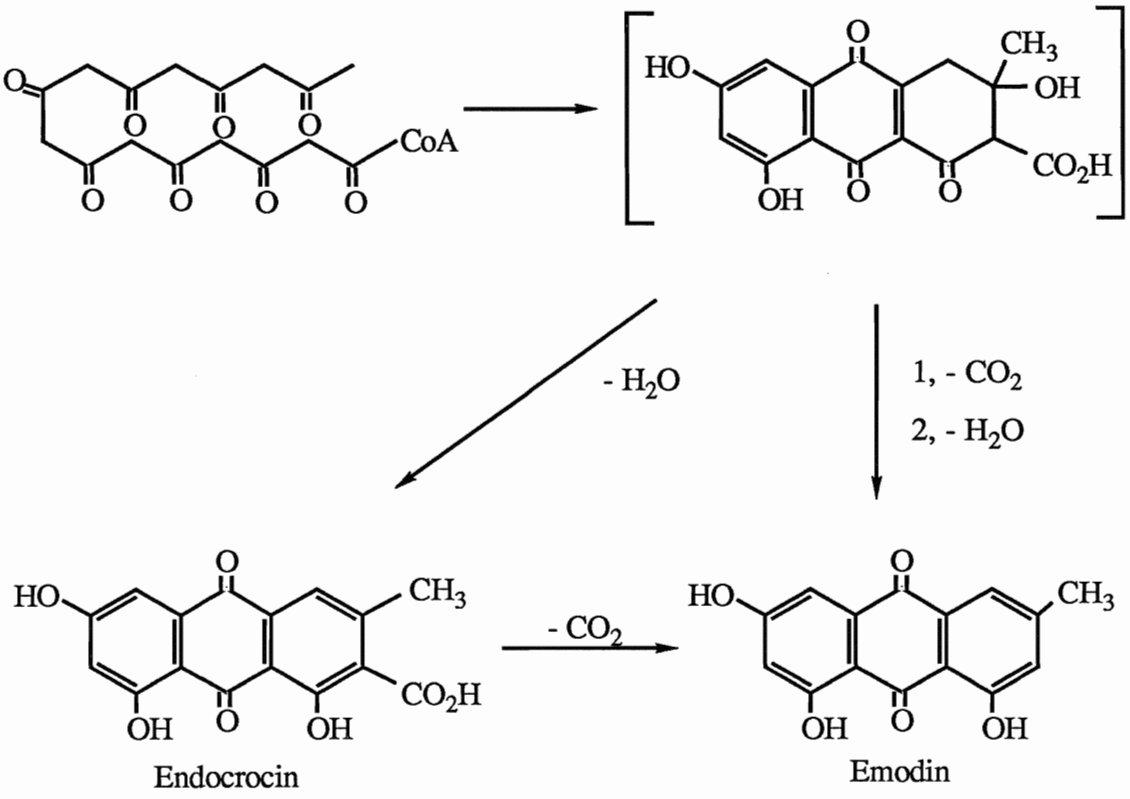


Scheme-5

The polyketide pathway leads to anthraquinones (Scheme-6). There are numerous anthraquinones produced by the octaketide pathway which conform to the basic emodin structure. They arise via different folding, O-methylation, side chain oxidation, nuclear

hydroxylation or elimination of hydroxyl groups, chlorination, dimerization via phenol oxidation, etc.

Polyketide pathway to anthraquinones:[25]



Scheme-6

II. ASYMMETRIC SYNTHESIS

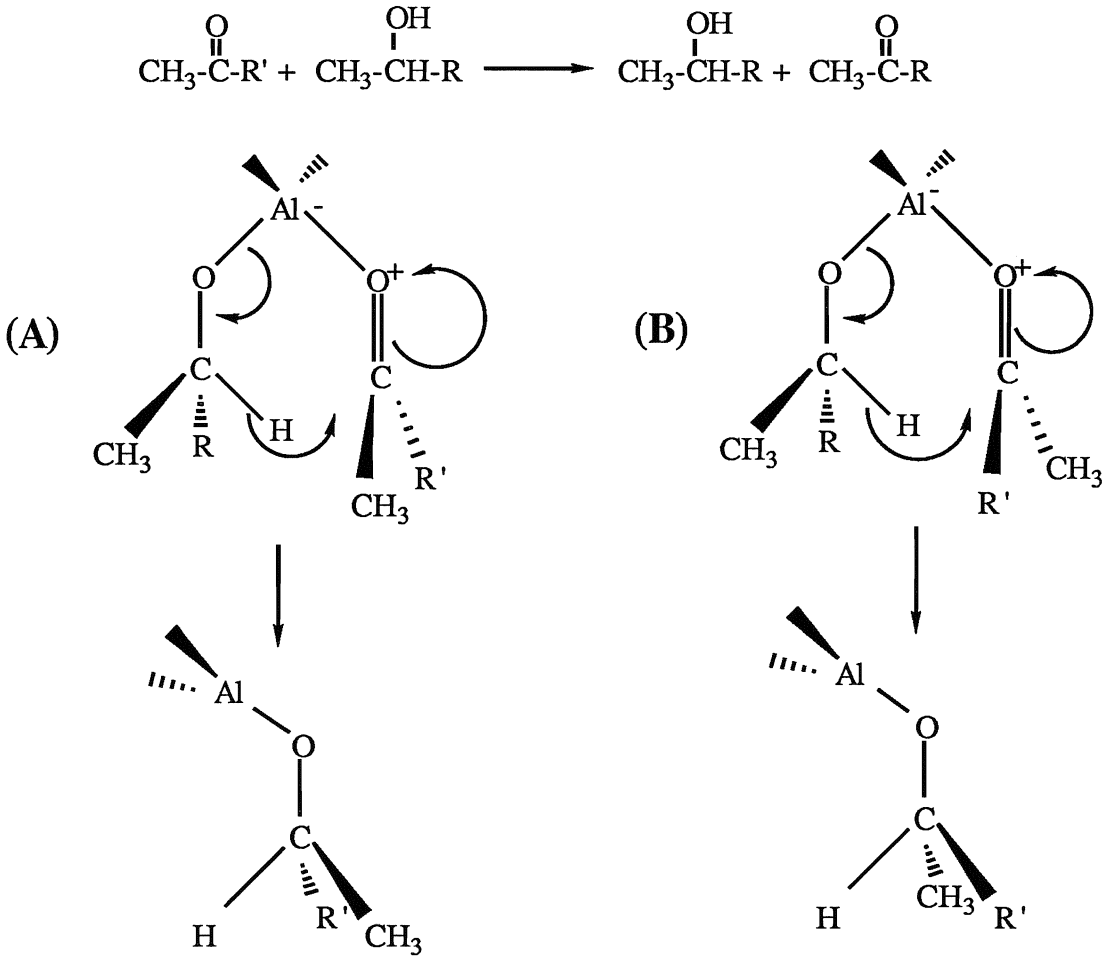
1. History

In 1894, Emil Fisher clearly outlined the concept of asymmetric syntheses based upon his experiments in the conversion of one sugar to its next higher homolog via a cyanohydrin reaction, relating this process directly to the biochemical process for the production of optical active sugars in plants.[26] He made the assumption that carbon dioxide and water condensed to give formaldehyde (Equation-9) under the influence of sunlight and chlorophyll.

Equation-9: $CO_2 + H_2O \rightleftharpoons H\text{-}\overset{\overset{O}{\|}}{C}\text{-}H + O_2$

Furthermore, he assumed that the formaldehyde condensed with itself and with simple carbohydrates under the direction of the optically active substances in the chlorophyll-containing granules in the cell. This occurred in such a way that the formation of each successive asymmetric carbon atom in the chain produced only one of the two possible stereoisomeric forms. As the reaction proceeded, a sugar molecule formed in close association with the chlorophyll. This formation was followed by separation of the optically active sugar and regeneration of the chlorophyll catalyst so that it was available to continue the cycle. This concept of asymmetric synthesis as envisaged by Emil Fischer is, in essence, valid today.

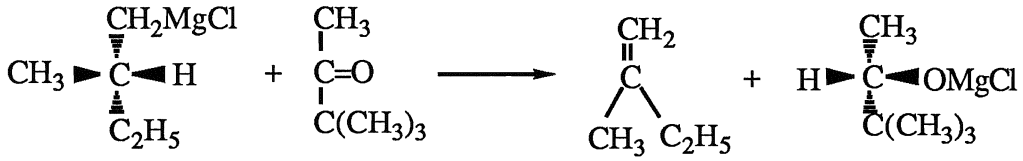
By 1949, the science of organic chemistry was ready for a succession of events that led to the interpretation of several key asymmetric reactions on a rational stereochemical basis founded upon conventional steric and electronic concepts. Doering and Young;[27] Jackman, Mills and Shannon;[28] and Baker and Linn[29] carried out the asymmetric Meerwein-Ponndorf-Verley reduction of a ketone with an optically active alcohol in the presence of aluminum alkoxide to give a secondary optically active alcohol. They interpreted these results in terms of steric interactions in the activated complex (Scheme-7).



Scheme-7

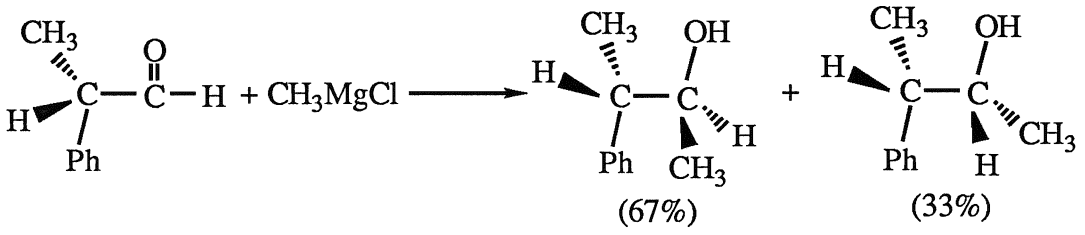
Vavon and his co-workers reported the first asymmetric Grignard reduction by using a terpene-derived Grignard reagent.[30,31] A systematic study of the asymmetric addition to ketones such as pinacolone of a chiral Grignard reagent to give an optically active alcoholate was done by Mosher and La Combe (Equation-10):[32]

Equation-10:



In 1952 Cram and Abd Elhafez published their results based on the stereochemistry of addition reactions of aldehydes or ketones having a chiral center next to the carbonyl group (Equation-11),[33] leading to the now well-known rule of steric control of asymmetric induction. In a three year period from 1949 to 1952, the foundations were laid for the rational interpretation of the stereochemical course of those organic reactions that had been classified as asymmetric synthesis. The period since the early 1950's has been one of exploring new systems and consolidating our knowledge in this area.

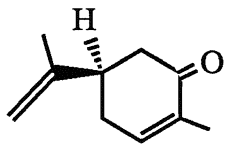
Equation-11:



2. Chirality in nature:

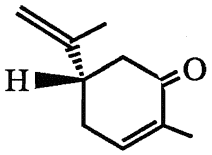
Compounds that occur in nature are optically active because living organisms tend to produce only a single enantiomer of a given molecule. The asymmetry of those molecules arises from the inherent chirality of the enzymes that are responsible for their productions. Receptor sites in biological systems, which are also optically active, have the ability to differentiate between two enantiomers of a specified molecule. Although the apparent physical differences between two enantiomers may seem small, the spatial orientation of a single functional group drastically affects the properties of the compound. This has strong implications even for the human body. For example; our nose can tell us the difference between the smells of (R)-carvone **6** and (S)-carvone **7;**

Carvone



(R)
spearmint odor

6

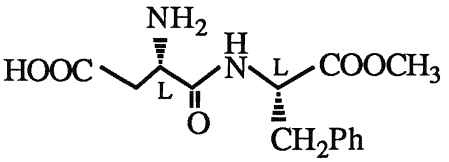


(S)
caraway odor

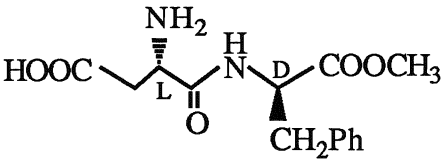
7

Aspartame is rapidly gaining an increasing market share as a low calorie sweetener and is used extensively in soft drinks, but its optical epimer has a bitter taste (Scheme-8).[34]

Aspartame



Sweet (L)

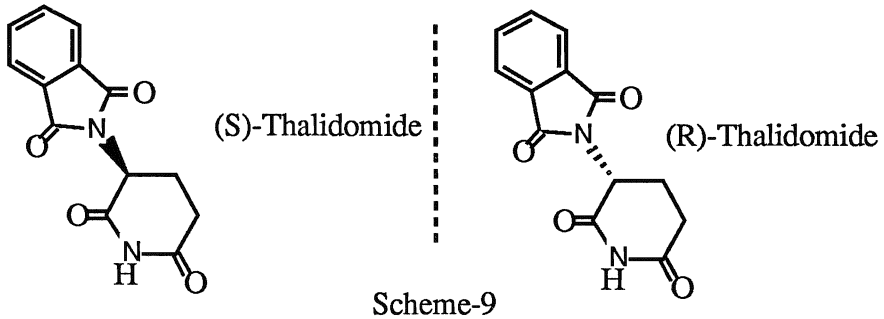


Bitter (D)

Scheme-8

Whenever a compound is introduced into the body, as either a food additive or a drug, the question of toxicity always arises. With molecules possessing one or more asymmetric centers, adverse toxicologic properties can sometimes be attributed to one stereoisomer and not the other.

In the early 1960's, thalidomide as a drug was administered in its racemic form for treatment. Its use by pregnant women resulted in a high incidence of fetal deaths, neonatal deaths, and congenital malformations.[35] The teratogenicity has subsequently been found to be the property of only the (S)-(-)-enantiomer (Scheme-9).[36]



Scheme-9

It is now common practice to at least synthesize and evaluate all the possible isomers of a new product before it is put into use.

3. Analytical methods: determination of enantiomeric purity

A. Polarimetric methods:

Most of the physical properties of the two enantiomers are identical. They have identical melting points, boiling points, solubilities in common solvents, densities, refractive indices, and spectra. However, they differ in one important respect - the way in which they interact with polarized light. Any material that rotates the plane of polarized light is said to be optically active. If a pure compound is optically active, the molecule is nonsuperimposable on its mirror image. If a molecule is superimposable on its mirror image, the compound does not rotate the plane of polarized light, it is optically inactive. If a molecule is not superimposable on its mirror image, it is chiral. If it is superimposable on its mirror image, it is achiral. The relationship between optical activity and chirality is absolute (theoretically). No exceptions are known.

If a compound causes the plane of polarization to rotate in a clockwise (positive) direction , it is said to be dextrorotatory. If it causes the plane to rotate in a counterclockwise (negative) direction, it is called levorotatory. The amount of rotation α is not a constant for a given enantiomer, it depends on the length of the sample vessel, the temperature, the solvent, the pressure (for gases) and the wavelength of light. The specific rotation $[\alpha]$ is

obtained by dividing α by the concentration, *c* expressed in g ml^{-1} solution) and by the length of the cell, *l* (expressed in decimeters). The wavelength of light used is given as a subscript and the temperature at which the measurement was made is given as a superscript.

$$[\alpha]_D^t = \frac{\alpha}{l\,c} \quad \text{(for solutions)}$$

$$[\alpha]_D^t = \frac{\alpha}{l\,d} \quad \text{(for liquids)}$$

If we know the value of $[\alpha]$ for the pure material $[\alpha]_{pure}$, the optical purity of the sample can be determined very easily by measuring its rotation,

$$\textit{percent optical purity} = \frac{[\alpha]_{obs}}{[\alpha]_{pure}} \times 100\%$$

and the optical purity is equal to the percent excess of one enantiomer over the other:

$$\textit{percent optical purity} = \textit{percent enantomeric excess}\ \text{(e.e.)}$$

$$= \frac{[R] - [S]}{[R] + [S]} \times 100\%$$

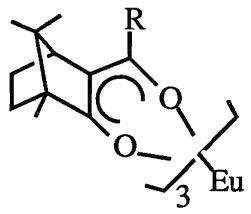
$$= \%\,R - \%\,S$$

B. Spectroscopic methods

Enantiomers can not be distinguished in an achiral medium by their spectra. However, in NMR spectra diastereomers may be distinguished because certain resonances are chemical shift non-equivalent. Determination of enantiomeric purity using NMR requires the intervention of a chiral auxiliary to convert an enantiomeric mixture into a mixture of diastereomers. Provided that the magnitude of the observed chemical shift non-equivalence is sufficient to give baseline resolution, integration of the appropriate signals gives a measure of the diastereomeric composition. This can be directly related to the enantiomeric composition of the original mixture. For this reason, there are three types of chiral auxiliary which have been widely used, chiral derivatizing agents, chiral solvent agents and chiral lanthanide shift reagents. Chiral derivatizing agents react with a pair of enantiomers to a pair of diastereomers which have different chemical shifts. Chiral solvents and chiral shift reagents form diastereomeric complexes via reversible equilibrium. An effective chiral auxiliary should induce significant NMR chemical shift anisochronicity in as large a range of substrates as possible.

The lanthanide shift reagents have the property of spreading NMR peaks of compounds with which they can form coordination compounds, e.g.: alcohols, carbonyl compounds etc. Chiral lanthanide shift reagents shift the peaks of the two enantiomers of many such compounds to different extents. Tris-[3-(heptafluoropropyl-

hydroxymethylene)-(1R)-camphorato] europium(III) **8** has been used extensively to measure the enantiomeric purity of chiral alcohols.



$R = CF_2CF_2CF_3$

Tris-(3-heptafluoropropyl-
hydroxymethylene-(1R)-camphorato)-europium(III)
$[Eu(hfc)_3]$

8

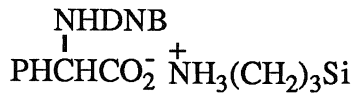
C. Chromatographic methods: GC and HPLC

The first attempts to systematize the chromatographic separation of diastereomers were made by Westley and co-workers (1968).[37] Westley's gas chromatographic separation of diastereomeric esters is now largely of historical interest as a foundation on which subsequent liquid chromatography has been built.

The separation of enantiomers requires the intervention of a chiral agent. This can take the form of long term derivatization of a pair of enantiomers with a chiral derivatizing agent to afford chromatographically separable diastereomers, or it can take the form of short-term interaction of the enantiomers with a chiral agent to afford short-lived diastereomeric complexes. The approach of the short-lived diastereomeric complexes is the most popular method to be used in recently years. This method involves a column

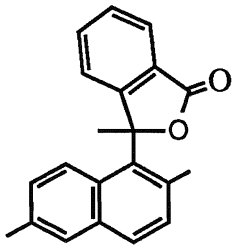
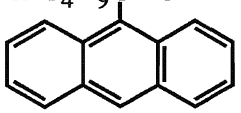
packed with a chiral agent (chiral stationary phases) which forms diastereomeric complexes with enantiomers, which will elute at different time.

Quartz, wool lactose and starch have been used for chiral stationary phases. Synthetic stationary phases are based on three interactions; hydrogen bonding, charge transfer (π-donor-π-acceptor based) and steric repulsive types. Compound **9** is one of the popular chiral stationary phases which has been used widely to separate racemic alcohols, esters, and amides. Table-2 shows two examples where compound **9** was used as a chiral stationary phase:[38]



9

Table - 2:

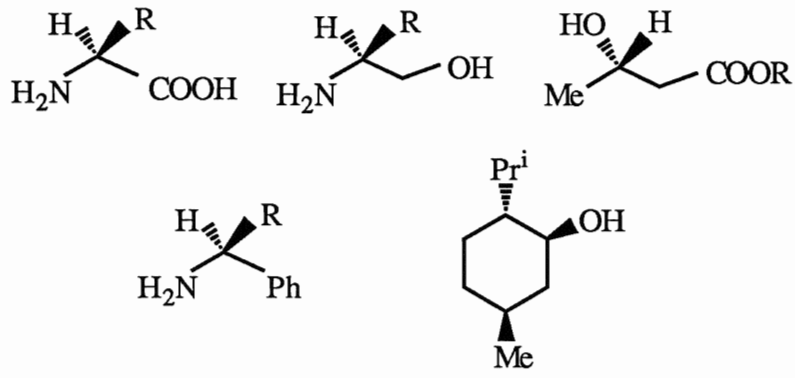
	Enantiomeric excess	
	First band	Second band
	> 99%	> 99%
n-C_4H_9CHOH 	97%	99%

(DNB: dinitrobenzoyl)

4. Asymmetric synthesis

The chiral compounds which occur in nature provide an enormous range and diversity of possible starting materials. To be useful in asymmetric synthesis, these should be cheap and readily available in high enantiomeric purity. For many applications the availability of both enantiomers is advantageous. Most importantly, they must be capable of exerting a high degree of stereocontrol in the required reactions by means of steric hindrance, chelation or other specific effects.

Naturally occurring amino acids, amino alcohols, hydroxy acids, terpenes, carbohydrates, alkaloids and other amines, etc. (Scheme-10), are the most widely used chiral starting materials for asymmetric synthesis.



Scheme-10

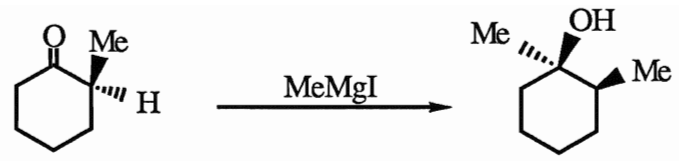
The known methods of asymmetric synthesis may be conveniently classified into four types according to how the enantiomerically pure compound is used.

A. *' First-generation'*

The 'first-generation' starts with an enantiomerically pure compound (almost invariably of natural origin) which is incorporated into the final product. The whole reaction may be represented as:

$$S - X^* \xrightarrow{R} P^* - X^*$$

This involves the formation of a new stereogenic centre in a substrate (S) under the influence of an adjacent stereogenic group (X*) already present. If the reagent is denoted by R, the product by P, and chirality by an asterisk. A specific example (Scheme-11) is provided by the addition of methyl reagents to (S)-2-methylcyclohexanone **10** to give 1(R),2(S)-1,2-dimethylcyclohexanol **11** in which addition to the carbonyl group is influenced by the adjacent stereogenic centre according to Cram's rule.[33,39]



10 **11**

Scheme-11

B. 'Second-generation'

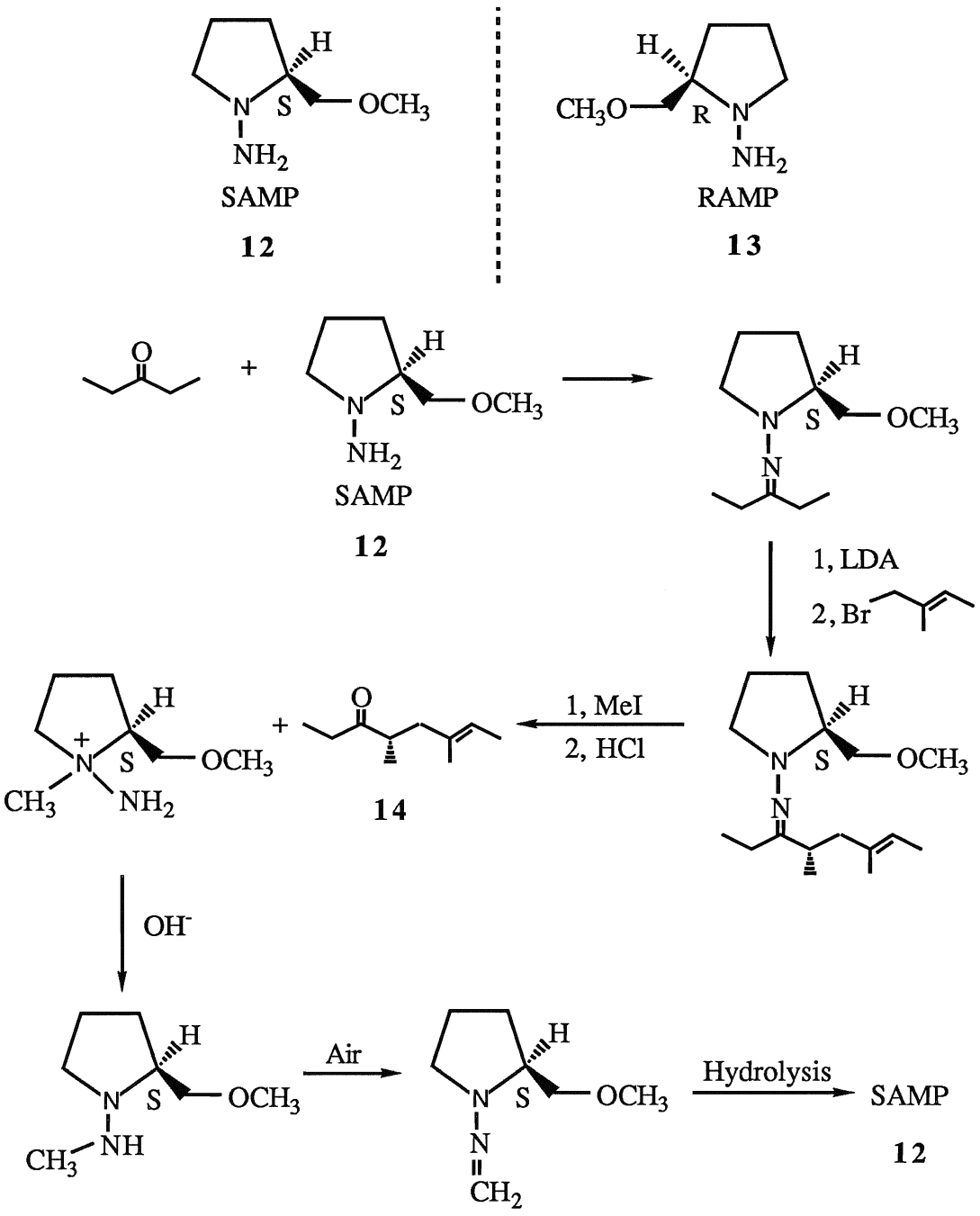
An achiral substrate is made chiral by attachment of a chiral auxiliary (A*), which then directs subsequent reaction and is finally removed to give the chiral product.

$$S \xrightarrow{A^*} S - A^* \xrightarrow{R} P^* - A^* \xrightarrow{-A^*} P^*$$

This method has one important advantage that the auxiliary can be recovered and recycled, but it also suffers from the disadvantage that two extra synthetic steps are required, one to introduce the auxiliary, and another to remove it. Most recently developed methods are of this type.

(R)-(+)-1-Amino-2-(methoxymethyl) pyrrolidine (RAMP) **13** are commercially available chiral auxiliary reagents that have been used successfully for the asymmetric alkylation of aldehydes and ketones. For example: 3-pentanone is converted via its SAMP (**12**)

hydrazone into the chiral (S)-ketone **14** which has an enantiomeric excess of better than 95% (Scheme-12).[40,41]



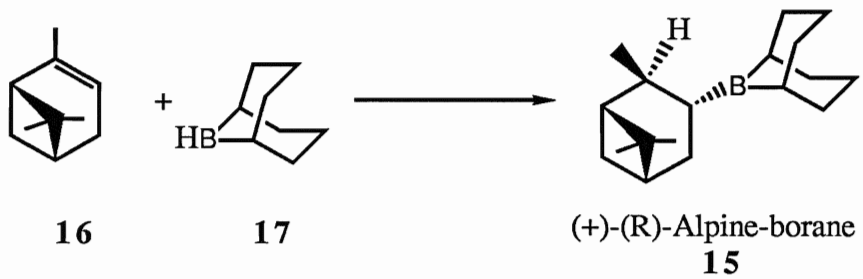
Scheme-12

C. 'Third-generation'

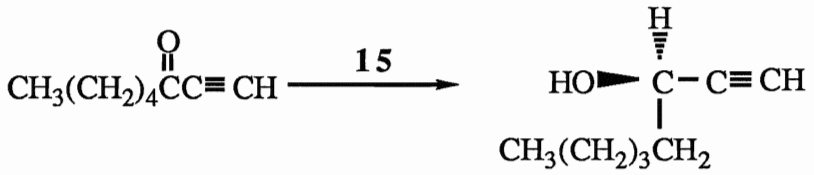
The attractiveness of the auxiliary approach may be enhanced by the use of a chiral reagent, which converts the achiral substrate directly into a chiral product.

$$S \xrightarrow{R^*} P^*$$

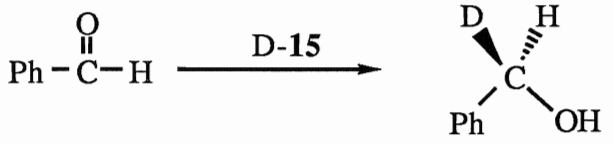
This method has the same disadvantage as the 'first-generation' method, in that an enantiomerically pure material is required.



16 **17** (+)-(R)-Alpine-borane **15**



90% e.e.



82% yield; 90% e.e.

Scheme-13

Alpine-borane® **15** is the delightful trivial name of the trialkylborane obtained from 9-borabicyclo-[3,3,1]-nonane **17** and (+)- or (-)-α-pinene **16**. It is commercially available reagent. It has been used to reduce a variety of ketones and aldehydes in high e.e. (Scheme-13).[42,43]

D. 'Fourth-generation'

A chiral product is produced by using a achiral starting material and a achiral reagent under a chiral catalyst.

$$S \xrightarrow[\text{Cat.*}]{R} P^*$$

Enzyme-catalyzed reactions are also included in this class. This method is the most attractive, since it is the most economical method because it uses only a small amount of chiral catalyst.

In recent years there has been a veritable renaissance in the field of chemical enzymology, due in part to the realisation that many enzymes will accept 'unnatural' substrates which can be used to probe their active sites. Enzymes are particularly valuable chiral catalysts because they can exert unique control over several stereochemical aspects in single step reactions, thereby enabling asymmetric transformations to be achieved that cannot currently be matched by nonenzymatic catalysts. Enzymes are exceptional in three main respects:

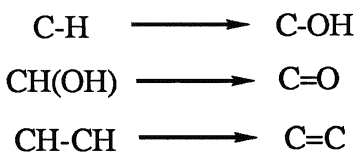
(1). They are extremely versatile and catalyzed reactions, and the reaction takes place under mild, often room temperature, and neutral pH, and conditions that minimize problems of isomerization, racemization, epimerization,and rearrangement that often plague traditional methodology.

(2). Enzymes are very effcient catalysts. The rates of enzyme-mediated processes can be faster than those of the corresponding nonenzymatic reactions by factors of up to 10^{12}.[44]

(3). Enzymes are generally very selective in terms of the type of reaction catalyzed, and with respect to the structure and stereochemistry of the substrate and product. These properties collectively constitute the specificity of an enzyme and are its most important features for asymmetric synthesis.

A wide variety of asymmetric reactions, including oxidation, reduction and hydrolysis, have been successfully performed, using either isolated enzymes or intact organisms such as yeast. Enzymes are classified into six main groups:[45]

(1). Oxidoreductases. Enzymes of this group catalyze oxidation-reduction reactions involving oxygenation, such as:



(2). Transferases. These enzymes mediate the transfer of groups such as acyl, sugar, phosphoryl, aldehyde and ketone from one molecule to another.

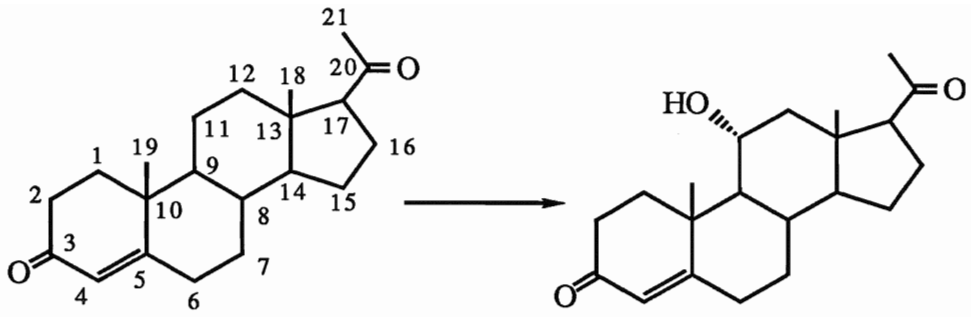
(3). Hydrolases. The range of functional groups hydrolyzed by such enzymes is very broad. It includes esters, anhydrides, glycosides, amides, and other C-N containing functional groups such as nitriles.

(4). Lyases. The reaction catalyzed are additions (usually of HX) to double bonds such as C=C, C=N and C=O, and reverse processes.

(5). Isomerases. A variety of isomerizations, including C=C migration, cis-trans isomerization and racemization, are effected.

(6). Ligases. These are ofen called synthetases. They catalyze formation of C-O, C-S, C-N, C-C and phosphate ester bonds.

One of the most dramatic early examples of the use of enzymes in chemical synthesis involves the formation of corticosteroids. Before 1950, the compound **19** was a key intermediate in the synthesis of the anti-inflammatory corticosteroids, for which a 30 step synthesis was needed. Most of these steps were taken up in introducing an oxygen at C-11 of the steroid skeleton. In 1952, the Upjohn group headed by O.H.Petersen succeeded in converting progesterone **18**, which is a cheap and abundant natural steroid, into its 11α-hydroxy derivative **19** in 90% yield using the fungus *Rhizopus arrhizus* (Scheme-14).[46]



(Progesterone)

18

19

Scheme-14

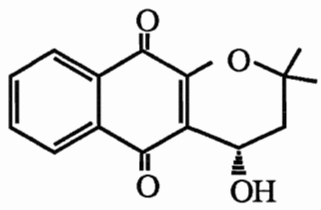
The studies of biotransformation have shown that the hydroxyl group can be introduced into a steroid molecule at any position and with almost any desired stereochemistry by microorganisms.[47] Table-3 shows some hydroxylations of steroid **18**:[48]

Table-3:

Microorganism	Site of attack	Yield (%)
Bacillus species	14α	36
Aspergillus fumigatus	7β+15β	18
Acremonium strictum	6β+11α	21
Botryosphaerica obtusa	7β	High
Sepedonium ampullosporum	16α	26
Phycomyces blakesleeanus	7α	34
Apiocrea chrysosperma	14α+15β	23

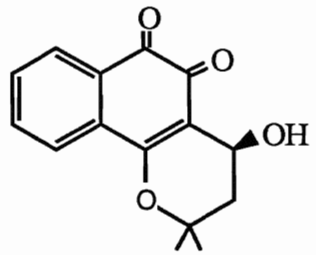
III. *(S)*-4-HYDROXY-α-LAPACHONE 20

(S)-4-Hydroxy-α-lapachone **20** has been shown to have antineoplastic potential.[49] It was first isolated from the wood of *Catalpa ocata* [50,51] (Japanese name "*Kisasage*") in 1974, and was also found in Brasil from the heartwood of *Zeyhera tuberculosa*.[49] The absolute configuration was determined to be *(S)* by applying the extended dibenzoate chirality rule to the CD spectra of the benzoate ester.[52]



(S)-4-Hydroxy-α-lapachone

20



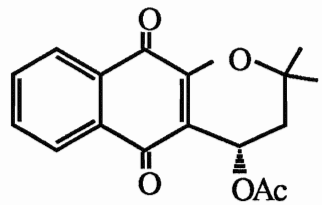
(S)-4-Hydroxy-β-lapachone

21

Its structure has been proved by an unambiguous synthesis. (±)-4-Hydroxy-α-lapachone has been prepared chemically.[53,54] The β-isomer **21** is also known to occur in nature, and to possess medicinal properties.[55]

There are varying reports of the physical properties of 4-hydroxy-α-lapachone. It has been reported as a yellow oil (natural

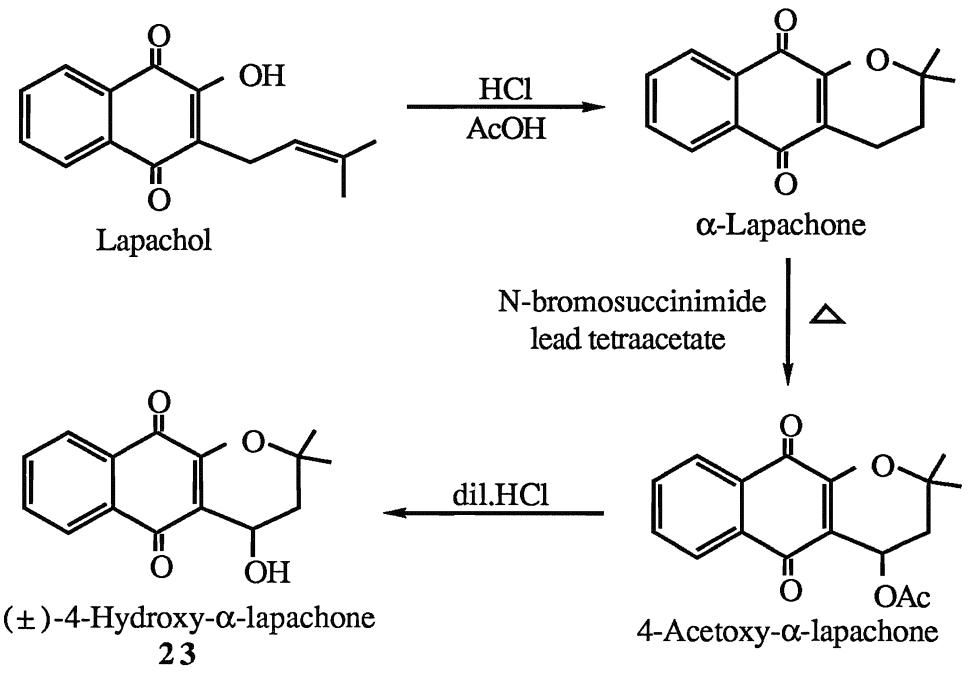
and synthetic),[49,50,51,54] and a yellow crystal (synthetic);[53] Its acetate **22** also has different m.p. reports: one was 118-120°C (natural),[49] others were 136-140°C natural,[50,51] and synthetic.[53,54] The optical rotations was: -OH $[\alpha]_D$= +27.4° (MeOH); -OAc $[\alpha]_D$= -14.2° (MeOH).



4-Acetoxy-α-lapachone

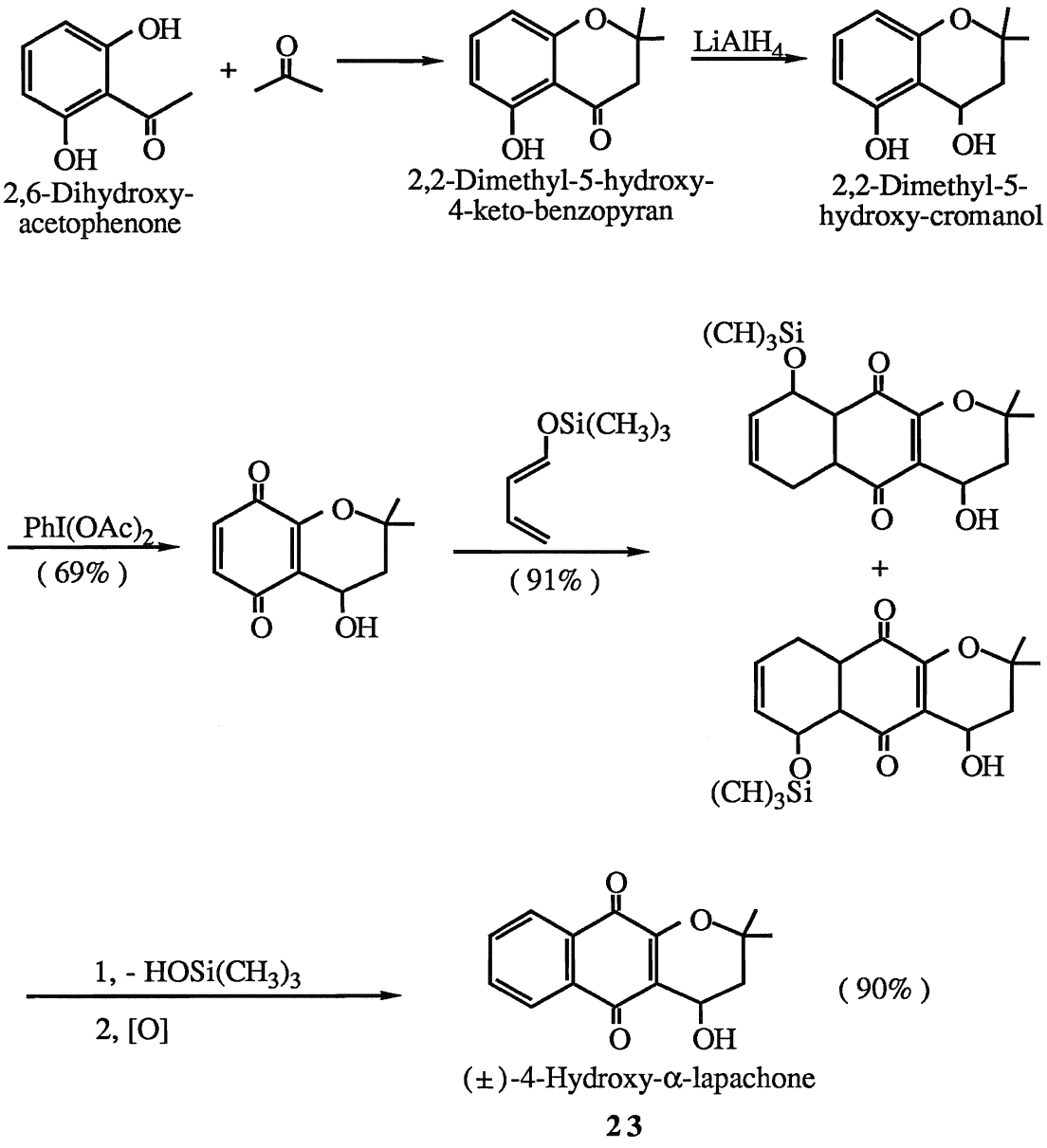
22

The first synthesis (Scheme-15) was published by Gupta and Khanna.[53] The natural product lapachol was the starting material. The synthesis involves the extension of the allyic acetoxylation which was first carried out by Barton,[56] and the use of N-bromosuccinimide and lead tetraacetate to give (±)-4-acetoxy-α-lapachone. The last step was hydrolysis to form (±)-4-hydroxy-α-lapachone **23.**



Scheme-15

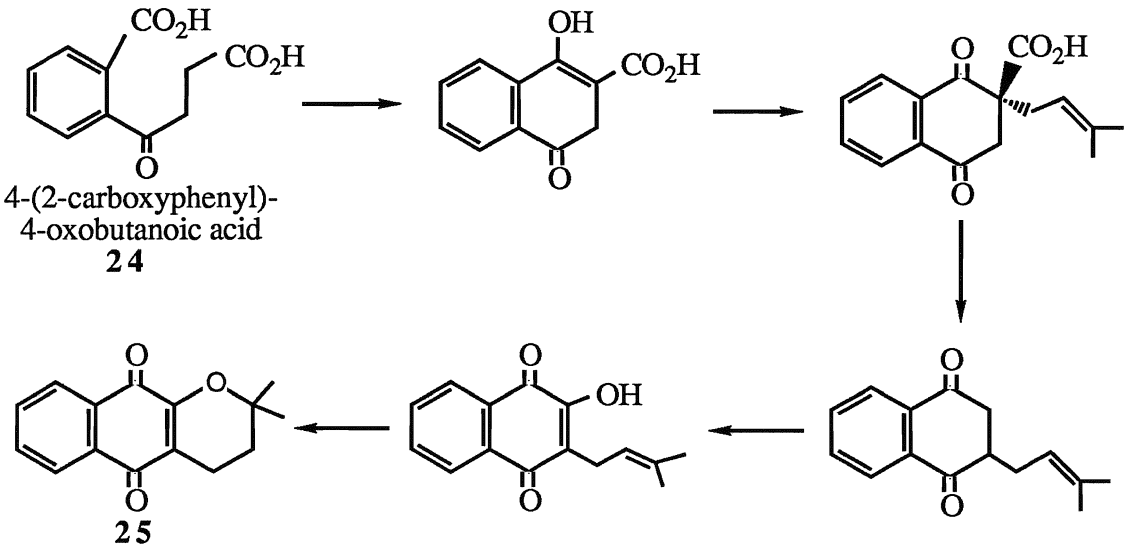
The total synthesis (Scheme-16) of (±)-4-hydroxy-α-lapachone **23** was also reported in 1992.[54] This synthesis started with 2,6-dihydroxy acetophenone, which was reacted with acetone in pyrrolidine to form 2,2-dimethyl-5-hydroxy-4-keto-benzopyran. Reduction to the alcohol was followed by oxidation to the quinone. A Diels-Alder cycloaddition reaction was then used to form the last ring. Finally, elimination of the siloxy group and oxidation lead to (±)-4-hydroxy-α-lapachone **23**



Scheme-16

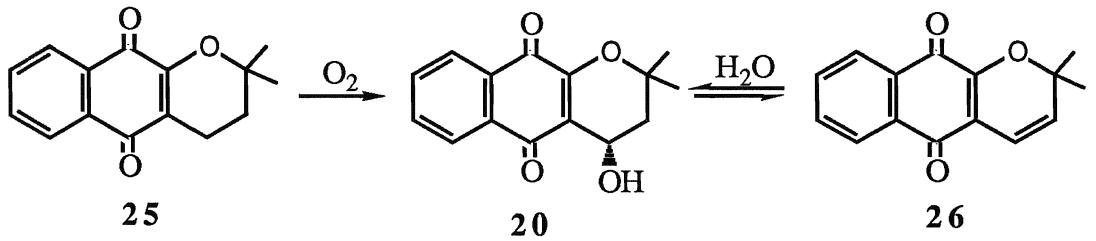
The biosynthesis of 4-hydroxy-α-lapachone has not been studied, but biosynthesis of catalpalactone derivatives in *Catalpa ovata*,[57] and the naphthoquinone[58] congeners in callus tissues which were induced from the seedlings of the plant and subcultured on the

Linsmaier-Skoog medium have been studied. The route shown in Scheme-17 has been proposed. 4-(2-Carboxypheny)-4-oxobutanoic acid **24** comes from shikimic acid (Scheme-5).



Scheme-17

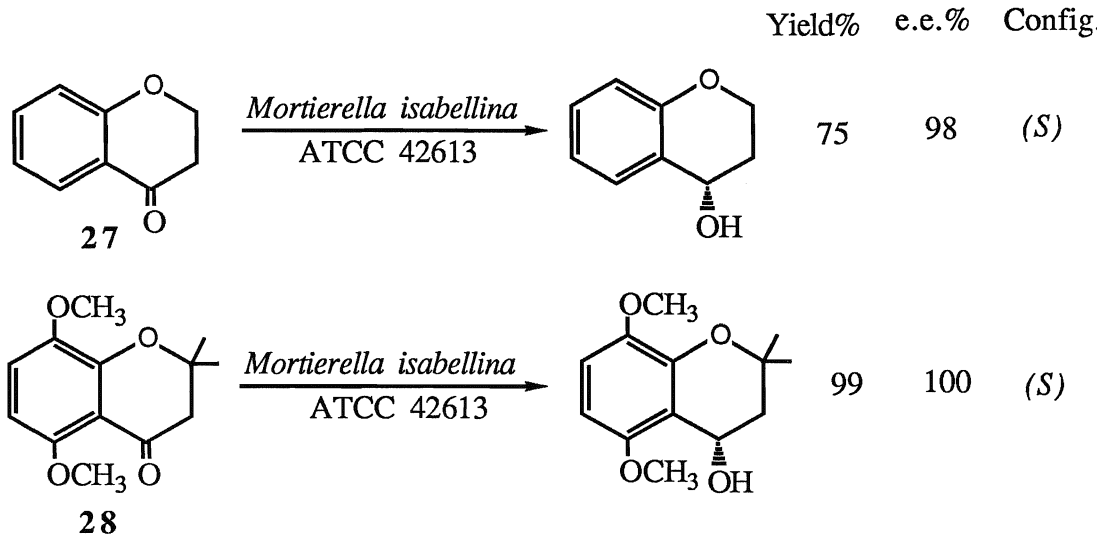
Natural *(S)*-4-hydroxy-α-lapachone has been isolated from the same sources as α–lapachone **25** and dehydro-α-lapachone **26**.[49,50,51] From this reason it is probable that 4-hydroxy-α-lapachone **20** is produced from α-lapachone by oxygenases, or from dehydro-α-lapachone by hydrolase activity (Scheme-18):



Scheme-18

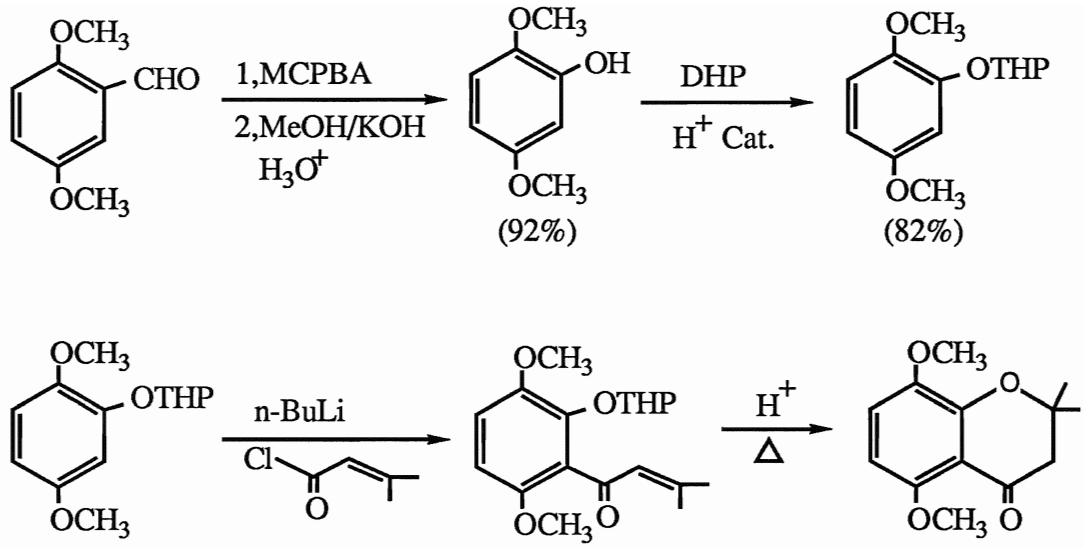
DISCUSSION

The principal difficulty in the stereoselective synthesis of 4-hydroxy-α-lapachone **20** is the reduction of a carbonyl group to an *(S)* alcohol. Recently, enzymatic reduction of 4-chromanone[59] **27** and 5,8-dimethoxy-2,2-dimethyl-chromanone[60] **28** has been done (Scheme-19). It shows very high yields with high optical purity. The absolute configuration of the two products was found to be *(S)*



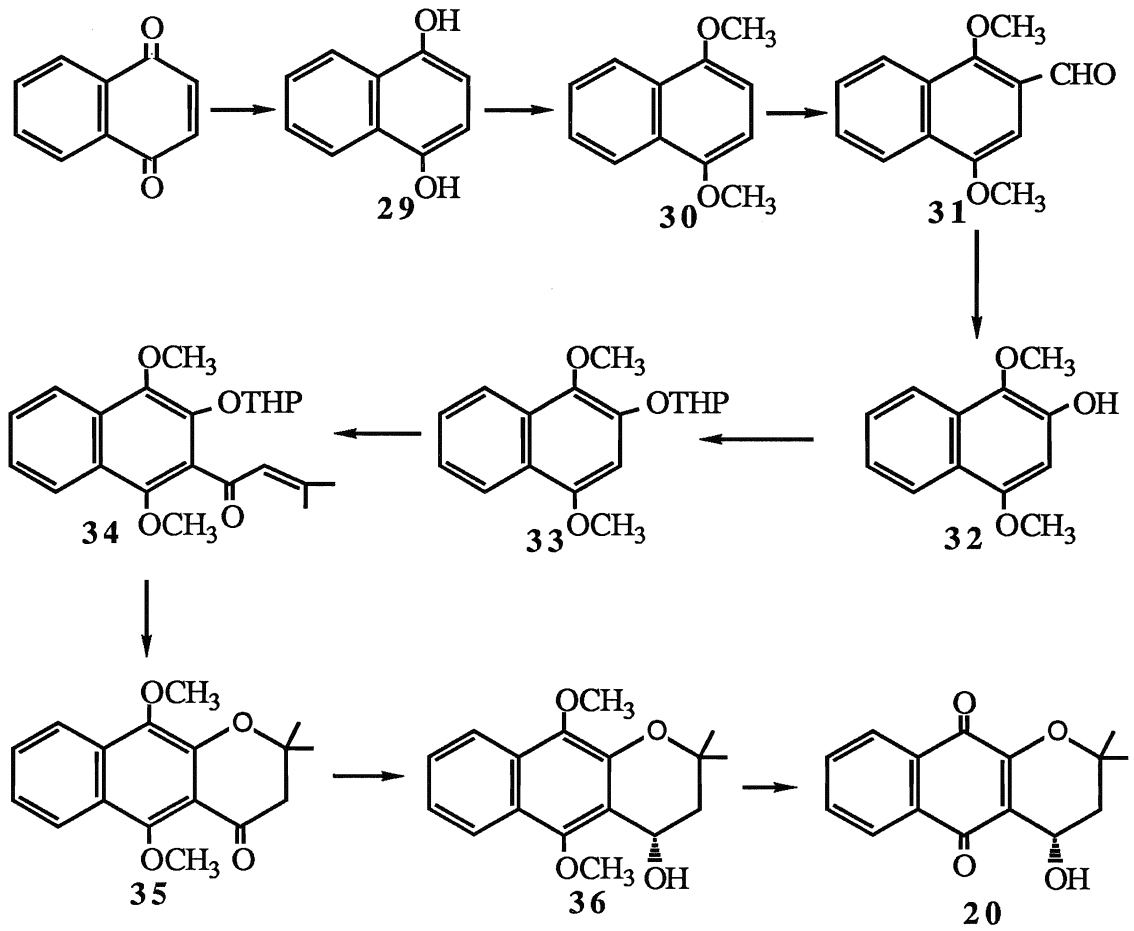
Scheme-19

The absolute stereochemistry of those products matches the configuration of our final product, based on the synthesis of 5,8-dimethoxy-2,2-dimethyl-4-chromanone **28** (Scheme-20) which has been done previously in this laboratory.[60]



Scheme-20

we proposed Scheme-21 for the synthesis of 4-hydroxy-α-lapachone **20**. The starting material 1,4-naphthoquinone can be reduced to 1,4-dihydroxynaphthalene **29**. A methyl ether was chosen to protect the phenolic hydroxyl groups because it can be easily cleaved by several reagents. Formylation and Baeyer-Villiger reaction would then be used to add a hydroxyl group at C-3, followed by protection by DHP. Lithiation followed by cyclization would then form the pyran ring, and the *(S)*-alcohol produced by biotransformation. Finally, compound **20** can be formed from compound **36** by using regiospecfic oxidation reagents such as silver(II) dipicolinate **5**.[15]



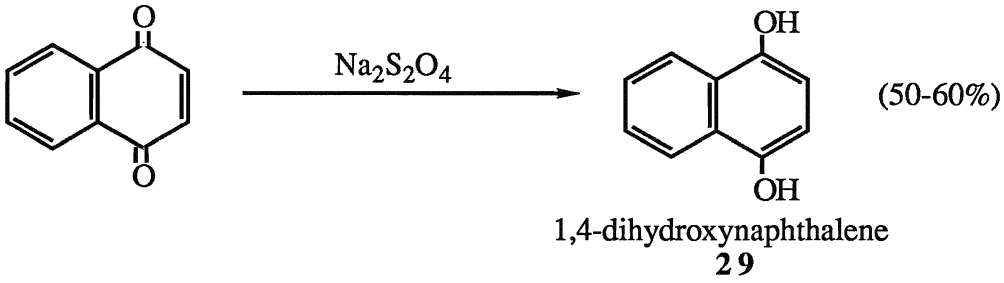
Scheme-21

I. THE FIRST APPROACH:

1. Preparation of 1,4-dihydroxynaphthalene **29**

As we know, quinones can be reduced easily to hydroquinones by many reduction reagents . Sodium hydrosulfite ($Na_2S_2O_4$) is the most well-known reagent for this purpose. 1,4-Dihydroxynaphthalene **29** was produced in this way from commercial 1,4-naphthoquinone. This hydroquinone **29** is not indefinitely stable in air, being reoxidized very easily to the quinone.

Equation-12

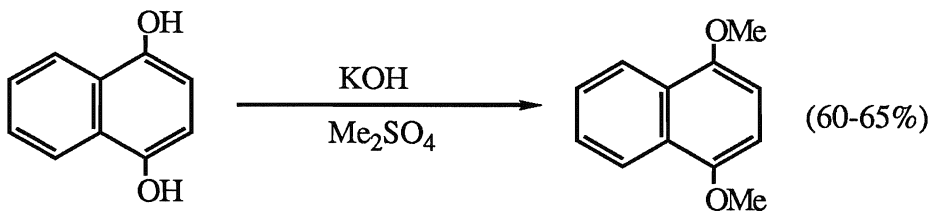


1,4-dihydroxynaphthalene
29

2. Preparation of 1,4-dimethoxynaphthalene **30**

The reactive phenolic hydroxyl group has to be protected to ensure success in later reactions. The methyl ether is a good protecting group because it is small and after formation, it is also very stable under most acidic or basic conditions. Generally methyl ethers can be prepared in a basic solution from a phenol and a halide or sulfate. Yield can be up to 74%.[61]

Equation-13



1,4-dimethoxynaphthalene
30

The procedure was simple for our product. The yield was not high, but the starting material can be recovered and reused.

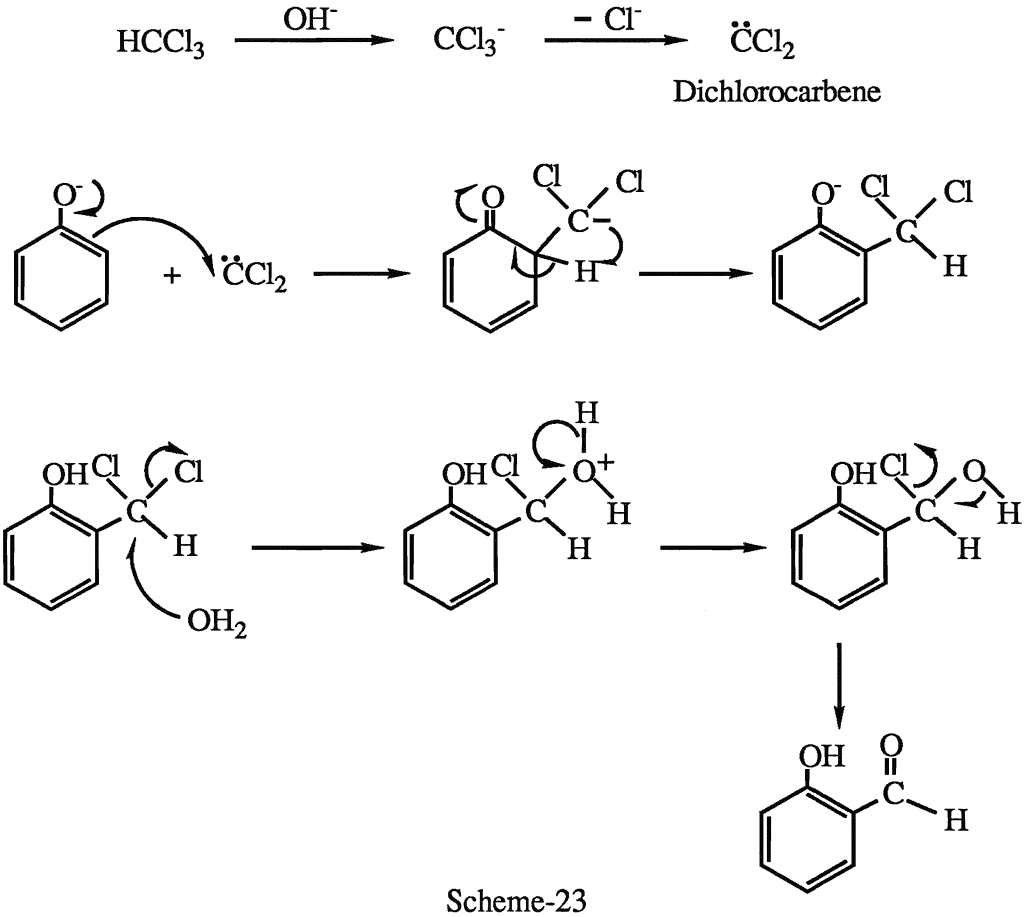
3. Preparation of 1,4-dimethoxy-2-naphthaldehyde **31**

There are many methods for electrophilic formylation of aromatic rings, but most of them require some kind of harsh conditions. The Gatterman formylation reaction can be applied to alkylbenzenes, phenols and their ethers (Scheme-22).[62] In the original version of this reaction the substrate was treated with HCN, HCl and $ZnCl_2$, but the use of $Zn(CN)_2$ and HCl (HCN and $ZnCl_2$ are generated in situ) makes the reaction more convenient to carry out. The mechanism of this reaction has not been thoroughly investigated.[63] It is better to avoid this method if there are other methods available, because of the toxicity of HCN.

Scheme-22

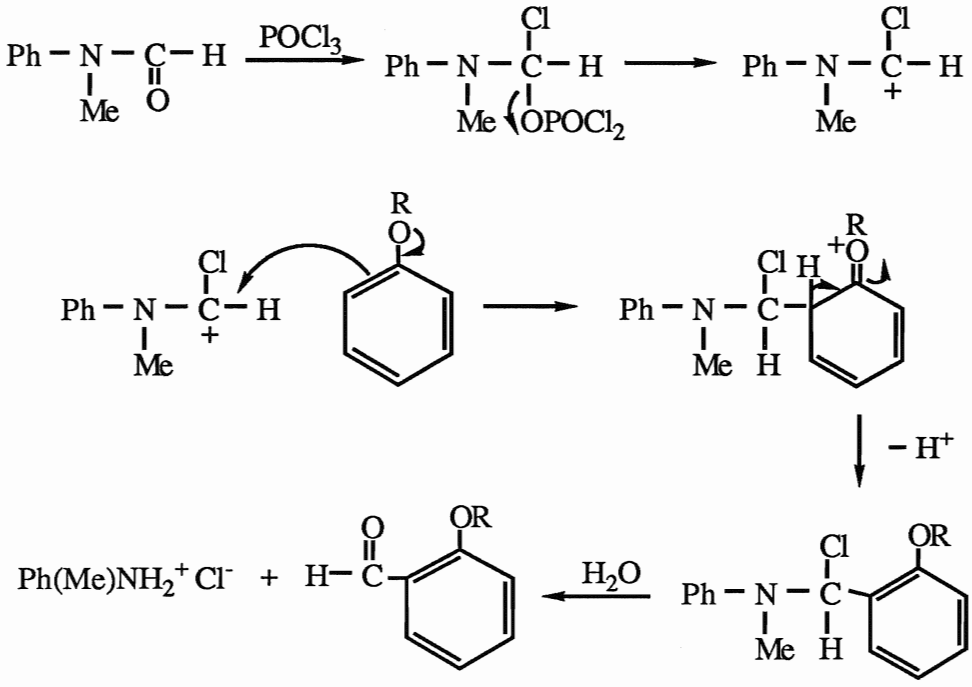
In the Reimer-Tiemann reaction chloroform and hydroxide ion are used to formylate the aromatic ring, but yields are normally low.[63] These reactions are only useful for phenols and certain heterocyclic compounds such as pyrroles and indoles. The first step is the loss of

a proton to give $^{-}CCl_3$ which then loses Cl^- to give dichlorocarbene, $:CCl_2$. The mechanism of this reaction is shown in Scheme-23:[64]



Scheme-23

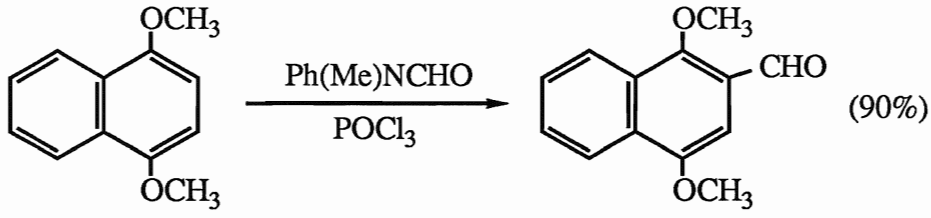
The Vilsmeier-Haack Reaction is the most common method for the formylation reaction, and was used in our procedure. This reaction is only applicable to aromatic rings which have electron donating substituents. The mechanism is believed to be that of Scheme-24:



Scheme-24

1,4-Dimethoxy-2-naphthaldehyde **31** was successfully prepared in 90% yield by reaction of 1,4-dimethoxynaphthalene **30** with N-methylformanilide and phosphorus oxychloride (Equation-14).[65]

Equation-14:

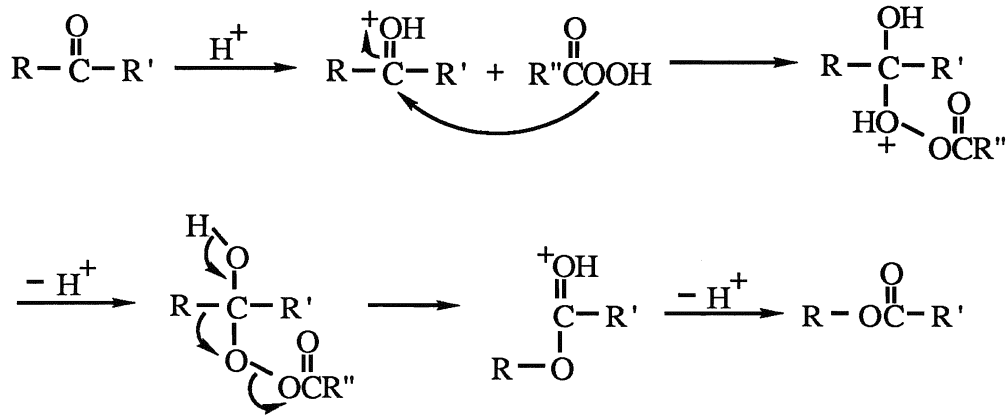


1,4-Dimethoxy-2-naphthaldehyde

31

4. The Baeyer-Villiger rearrangement reaction

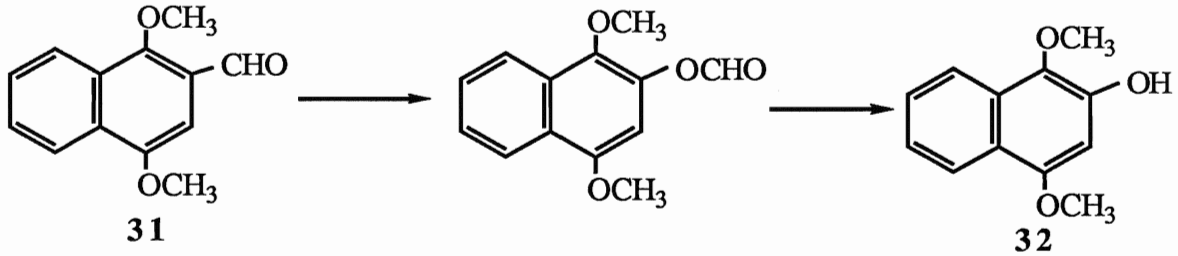
The treatment of ketones or aldehydes with peracids such as perbenzoic acid or peracetic acid, or with other peroxy compounds in the presence of acid catalysts, gives carboxylic esters by insertion an oxygen atom. This reaction is called the Baeyer-Villiger rearrangement. The mechanism is as follows (Scheme-25):[66]



Scheme-25

The approximate order of migration is tertiary alkyl > secondary, alkyl, aryl > primary alkyl > methyl. With aldehydes, migration of hydrogen gives the carboxylic acid. Migration of the other group would give formates, but this seldom happens, though aryl aldehydes have been converted to formates with variety reagents such as H_2O_2[67], and m-chloroperbenzoic acid (MCPBA).[65,68]

The compound **32** was produced with 45% yield by using this procedure. Other oxidation reagents (such as H_2O_2 and peracetic acid) were also used but yields were not higher than 50%.

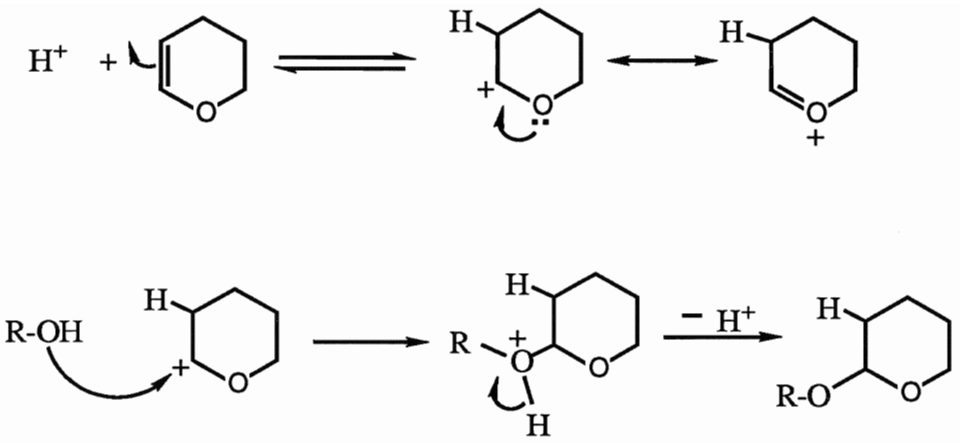


31 **32**

Scheme-26

5. Preparation of tetrahydropyranyl (THP) ether **33**:

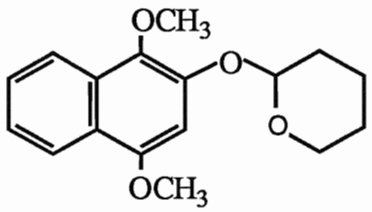
The acidic hydroxyl groups of alcohols or phenols have to be protected for reactions which are conducted in basic media. THP has been commonly used to protect both aromatic and aliphatic hydroxyl groups (Scheme-27). The hydroxyl can be regenerated with dilute acid.



Scheme-27

The yields from aliphatic alcohols are generally high (70-100%).[69,70] However, The yields from phenols vary from 20% to 83%.[71,72] Concentrated HCl and HOAc/EtOAc have been used as catalysts for the formation of THP ethers.[71,72]

In the event, we tried both reagents as catalysts for the formation of THP ether. The result was disappointing, because the product **33** and large amount of unreacted dihydropyran were obtained.

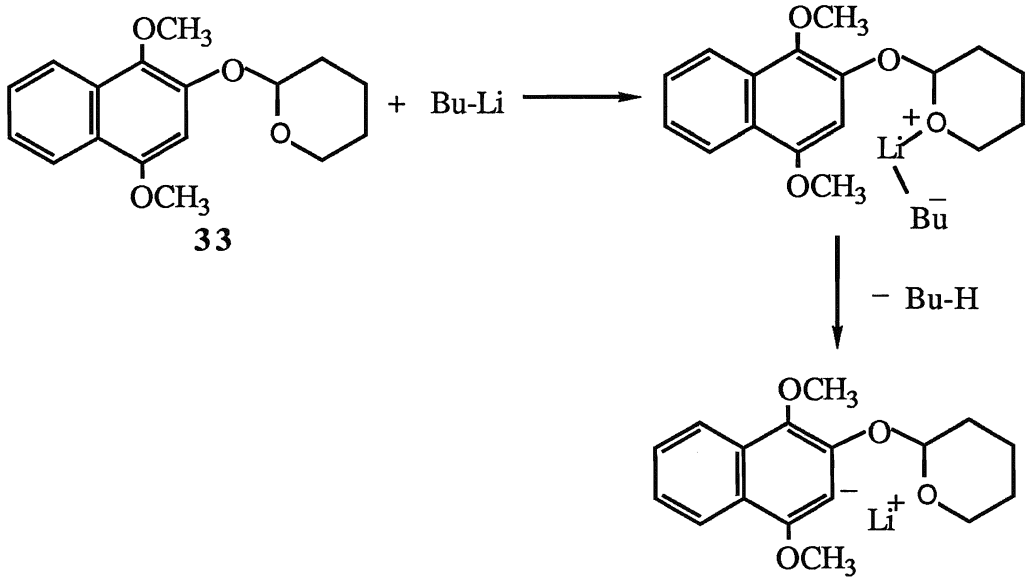


33

Chromatography and Kugelrohr distillation were used to purify the crude product, but with unsuccessful results. Because of the difficulty of purification, and the fact that crude product cannot be used for the next reaction, an alternative protecting group was considered.

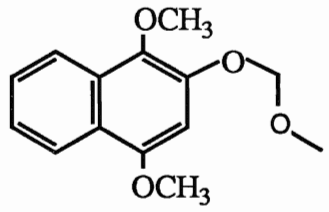
II. THE SECOND APPROACH:

What kind of protecting group should be used as the alternative? It has to have the same protecting properties, and also give a product may be easier to purify. In the first approach, THP was chosen not only as a protecting group, but also as a directing group for the next lithiation reaction. In Scheme-28 the oxygen coordinates with Li+ and promotes deprotonation of C-3 to form a carbanion.



Scheme-28

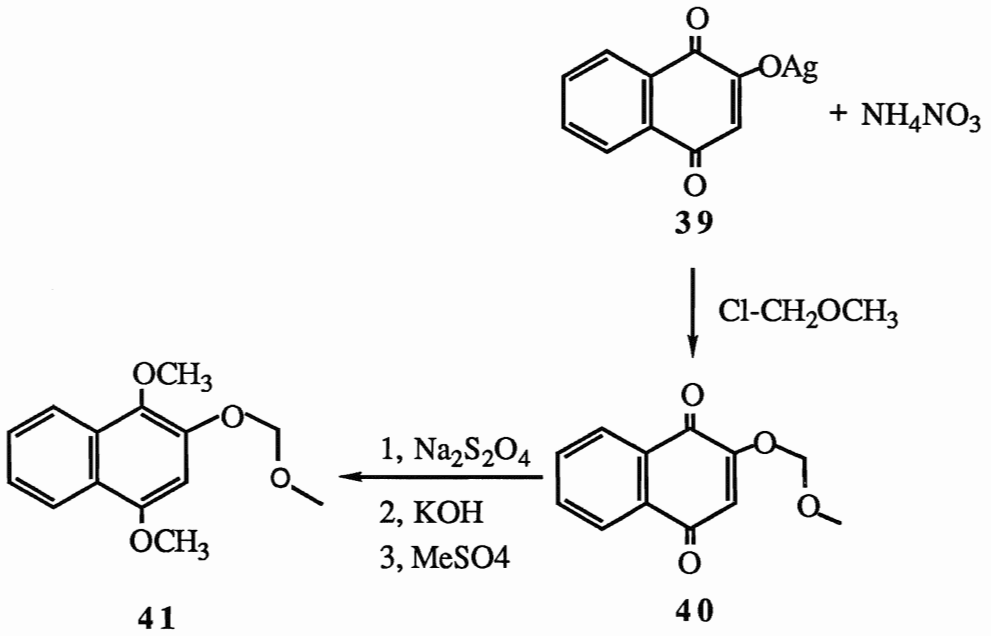
Methoxylmethyl (MOM) ether has the same acetal functional group with same directing effect, but less steric hindrance than THP. Therefore, MOM is the perfect protecting group for our reaction.



41

1. Preparation of 1,4-dimethoxy-2-(methoxymethoxy)-naphthalene **41**:

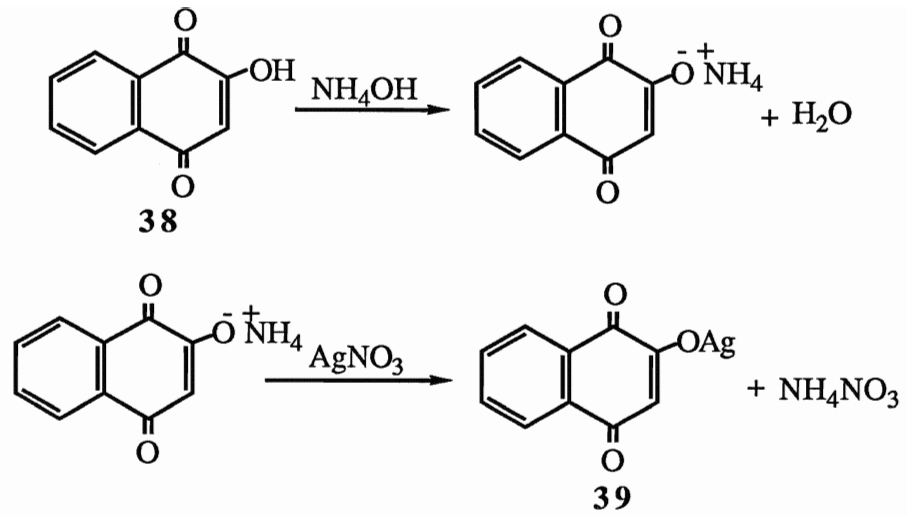
Compound **41** has been prepared by Kraus in 1979 (Scheme-29).[73] The procedure involved: alkylation of the silver salt of lawsone (**39**) with chloromethyl methyl ether to give the 1,4-naphthoquinone **40**. Reductive methylation of **40** with alkaline sodium dithionite and dimethyl sulfate then provides compound **41**.



Scheme-29

A. Preparation of the silver salt **39**:

The silver salt was first prepared by Miller,[74] and this method was developed by Fieser (Scheme-30).[75] The natural product lawsone **38**, which was the starting material, acts as an acid in reaction with excess NH_4OH to form a water soluble ammonium salt. The silver salt was then precipitated in high yield by adding $AgNO_3$.



Scheme-30

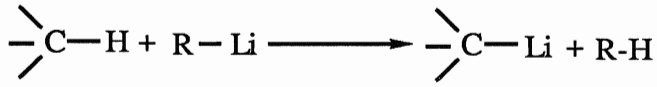
B. Preparation of compound **41**:

Preparation of 1,4-dimethoxy-2-(methoxymethoxy)-naphthalene **41** from the silver salt of lawsone was published in 1979 by Kraus.[73] The procedure was directly used for our proposal (Scheme-29). The silver salt **39** was convered to its MOM ether by reaction with chloromethyl methyl ether in 42% yield (Lit.:[73] 53%). The unreacted lawsone can be removed with 6N NH_4OH and 1N NaOH, and reused after acidification. The quinone **40** then was reduced to hydroquinone by sodium dithionite, followed by methylation with dimethyl sulfate. The yield of the product **41** was only 26% (Lit.:[73] 82%). At this time, we did not spend time to improve the yield, because there was enough product for trial of the next step.

2. Lithiation:

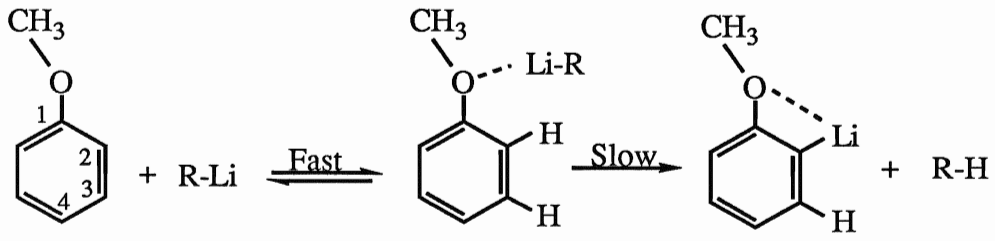
Lithiation is the replacement of a hydrogen atom by lithium to form a covalent lithium-carbon bond (Equation-15). This method can be used to form a new carbon-carbon bond for our synthesis. n-Butyl lithium and t-butyl lithium are the most important organolithium compounds.[76]

Equation-15:



Two mechanisms for this exchange have been proposed: acid-base and coordination (Equation-16).[77] In the intermediate coordinated species the carbon-lithium bond of the metalating agent and the carbon-hydrogen bond of the substrate are both polarized to some extent, thus allowing the more acidic 2-proton to be removed.

Equation-16:

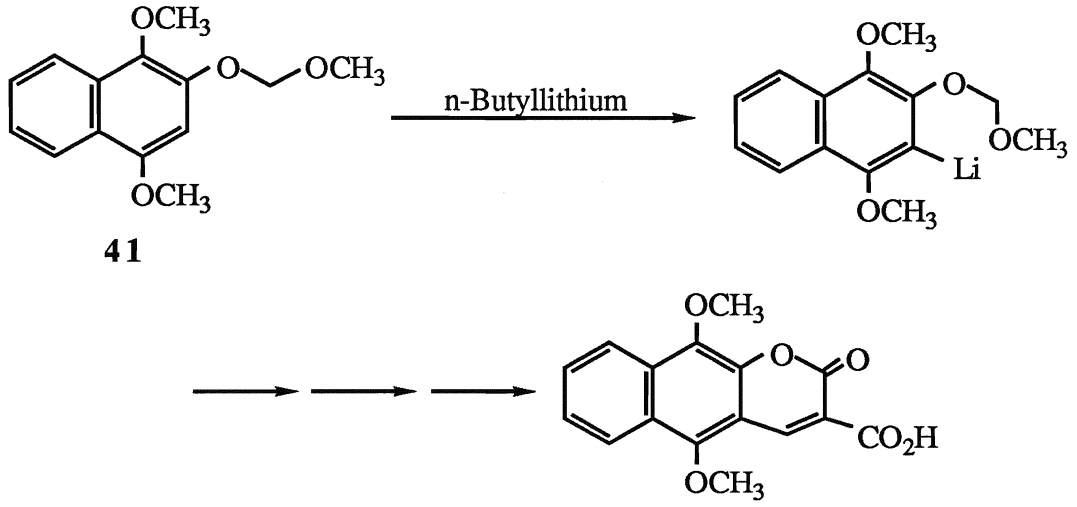


Phenyl lithium, methyl lithium, n-butyl lithium, and t-butyl lithium are all commercial available reagents which have been well studied. Table-4 shows pKa of the conjugate acids of these compounds:

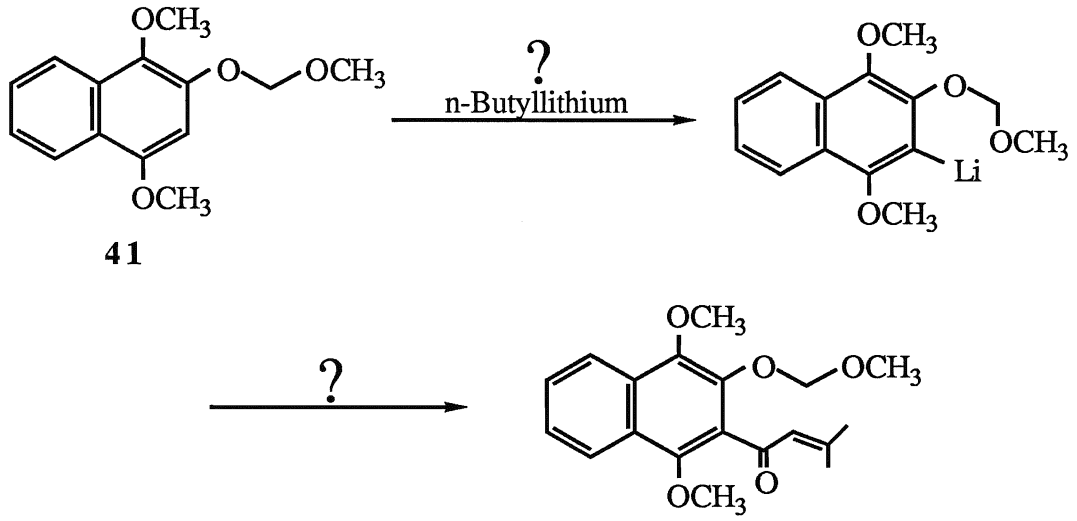
Table-4:

COMPOUND	pKa	Refs.
Benzene	37	78
Methane	40	78
n-Butane	45-50	79
iso-Butane	71	80

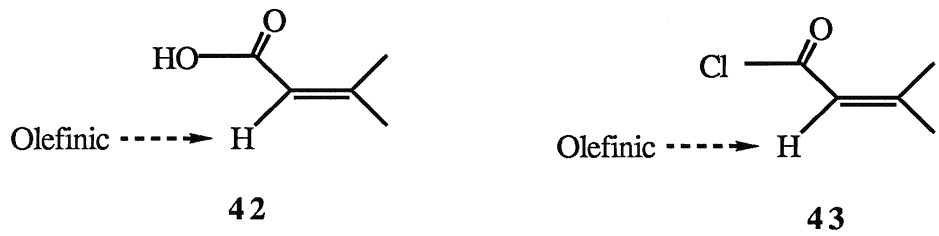
Kraus also used n-butyl lithium, which reacted with compound **41** in his procedure (Scheme-31). Because of the similarity of two reactions, the procedure was used in our reaction (Scheme-32), but only recovered starting materials were obtained and 3,3-dimethyl acrylic acid.

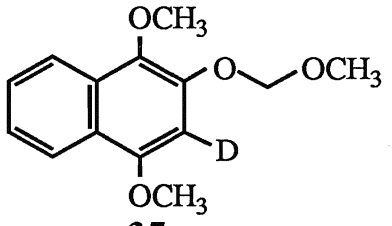


Scheme-31



Scheme-32





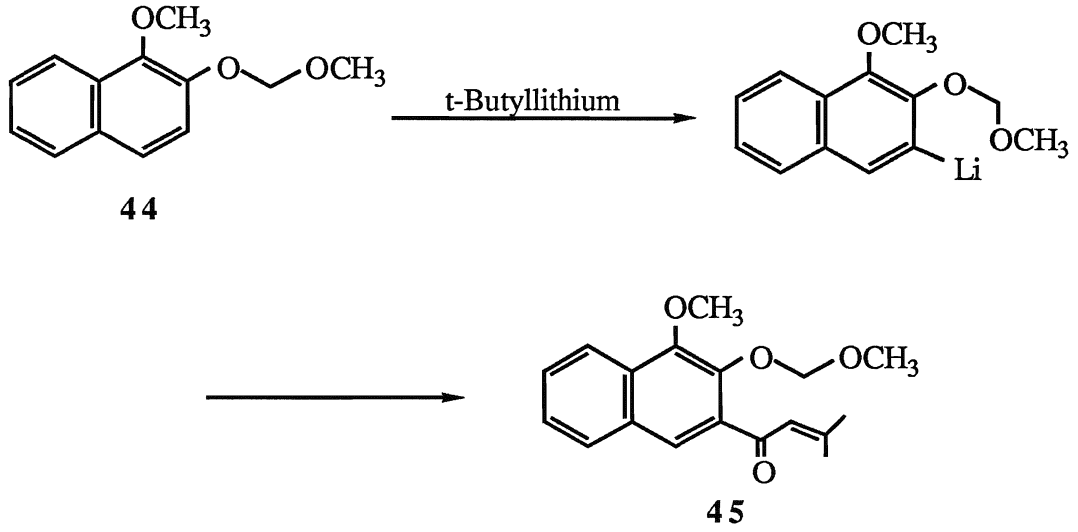
37

There were two possible reasons for this unsuccessful reaction: (1), the lithiation may not proceed; (2), Because of steric hindrance, the substrate may be too large for the reaction. D_2O was then used to examine the first possible reason. It was found that H-3 was not replaced by n-butyllithium in ether, but that using the stronger base, t-butyllithium H-3 was substituted by D giving compound **37**. For this reason, t-butyllithium was then used in the reaction instead of n-butyl lithium, but the reaction was still unsuccessful. This suggested that 2,2-dimethyl acryloyl chloride may have a large steric effect on the substitution.

The result told us that the structures of one of the two reactants would have to be changed. Manoharan's previous work showed that compound **43** was the only one of 7-8 substrates which added to an aryl lithium to give a ketone in a good yield.[60] It was unnecessary to spend more time on compound **43**. But it seemed worth to spend more time on compound **41**.

III. THE THIRD APPROACH

Considering compound **41**, removal of one methoxy group would possibly decrease the steric hindrance. Therefore, compound **44** was our next goal.

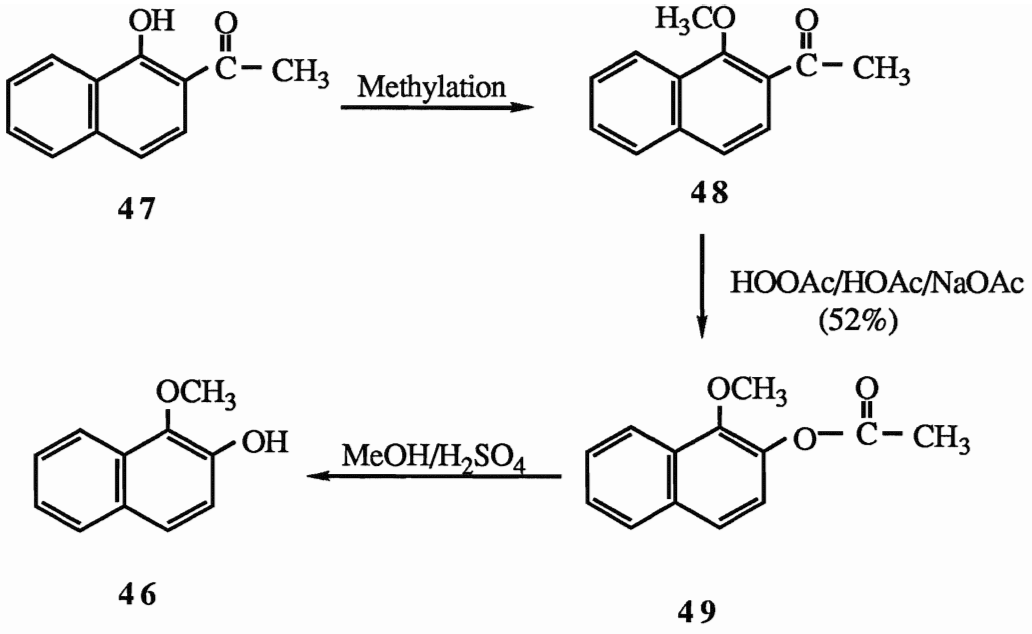


44 **45**

Scheme-33

1. Preparation of 1-methoxy-2-naphthol **46**.

There was a report for synthesis of 1-methoxy-2-naphthol **46** (Scheme-34).[81] It started with 1-naphthol to yield the 2-acetyl-1-naphthol **47** which is now a commercially available compound. The synthesis involved the methylation of the OH group, followed by Baeyer-Villiger oxidation and hydrolysis of the acetate **48** to give compound **46**.



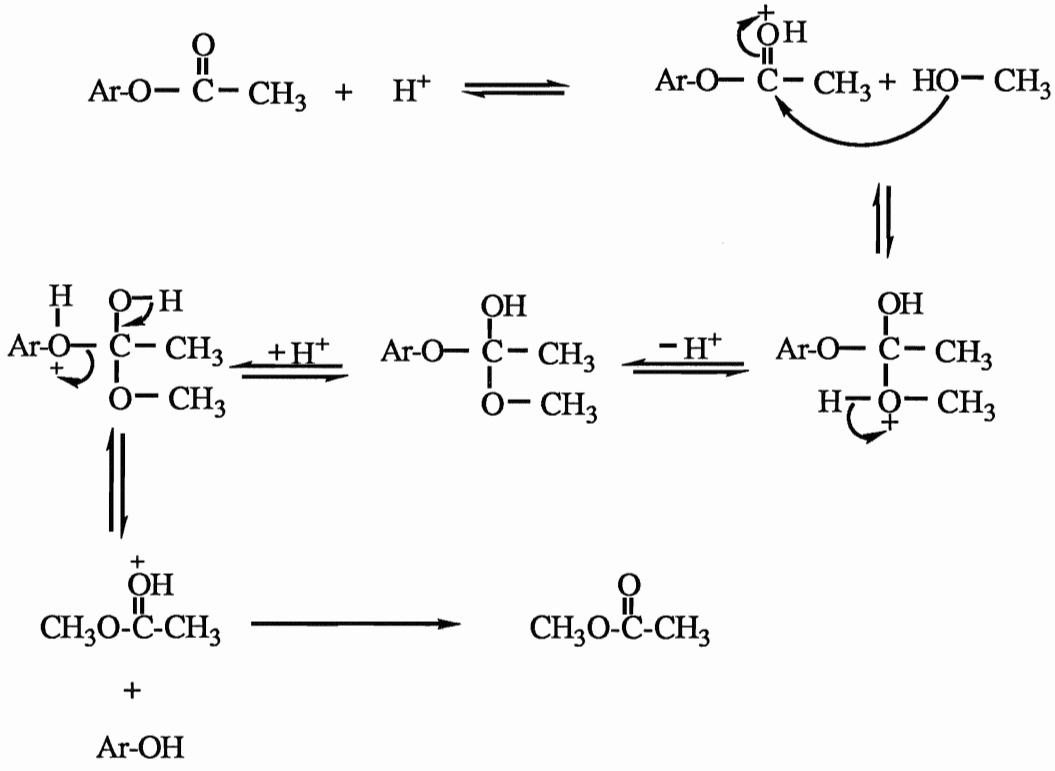
Scheme-34

There were no detailed procedures for methylation of **47** and hydrolysis of the acetate **49** in the report. These procedures may be general methods, but there was difficulty in methylating hydroquinones in our previous syntheses. We repeated the same procedure carefully,[61] with heated EtOH solution as hot as possible to make sure that all the phenol was in the solution, and also keeping the solution at the same temperature during the addition period. The yield was unexpectedly high, 96%. For this reason, solubility seems to be the most important feature in this reaction.

Peracetic acid was used for the Baeyer-Villiger oxidation reaction. The required temperature of the reaction was 41°C, and so dichloromethane which has boiling-point at 40°C, was used as a

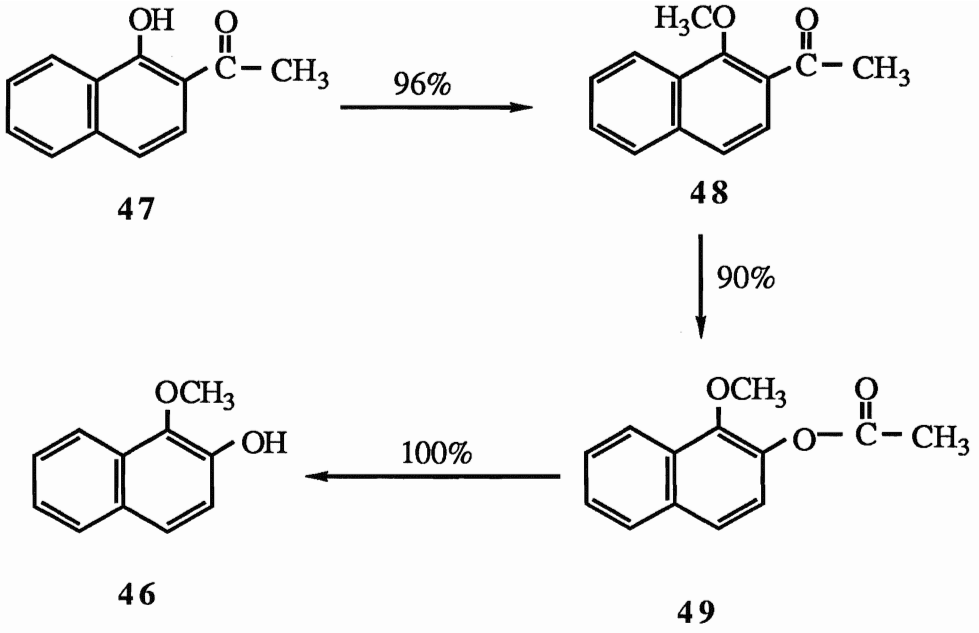
constant temperature bath. The yield was also high 90% (Lit[81] 52%). Fresh peracetic acid, and lower temperature may cause a more complete reaction.

Hydrolysis of the acetate in methanol and sulfuric acid was a very successful reaction. In this reaction, sulfuric acid acts as a catalysis. A large excess of methanol was used to replace the naphthol to give methyl acetate. The mechanism is shown in Scheme-35:



Scheme-35

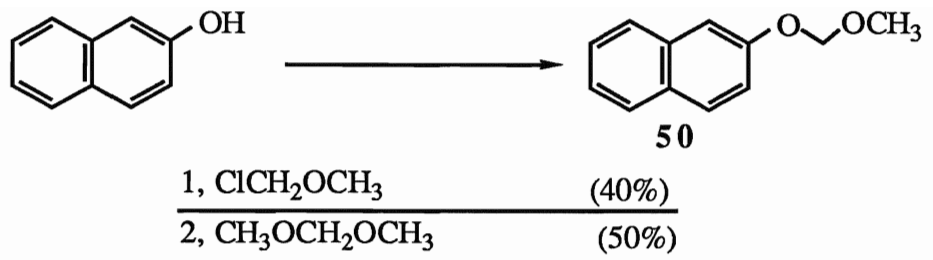
The overall yield was 86% for the three steps (Scheme-36).



Scheme-36

2. Protection of the hydroxyl group

In searching for the most covenient and efficient method for protecting a phenol group, we found general procedures for protecting the -OH group with MOM by using chloromethyl methyl ether or dimethoxy methoxy ether.[82] At this time, we used 2-naphthol as a model for this purpose and the results are shown in Scheme-37.



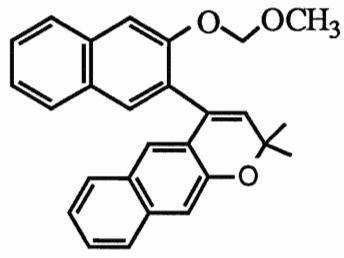
50

1, $ClCH_2OCH_3$ (40%)

2, $CH_3OCH_2OCH_3$ (50%)

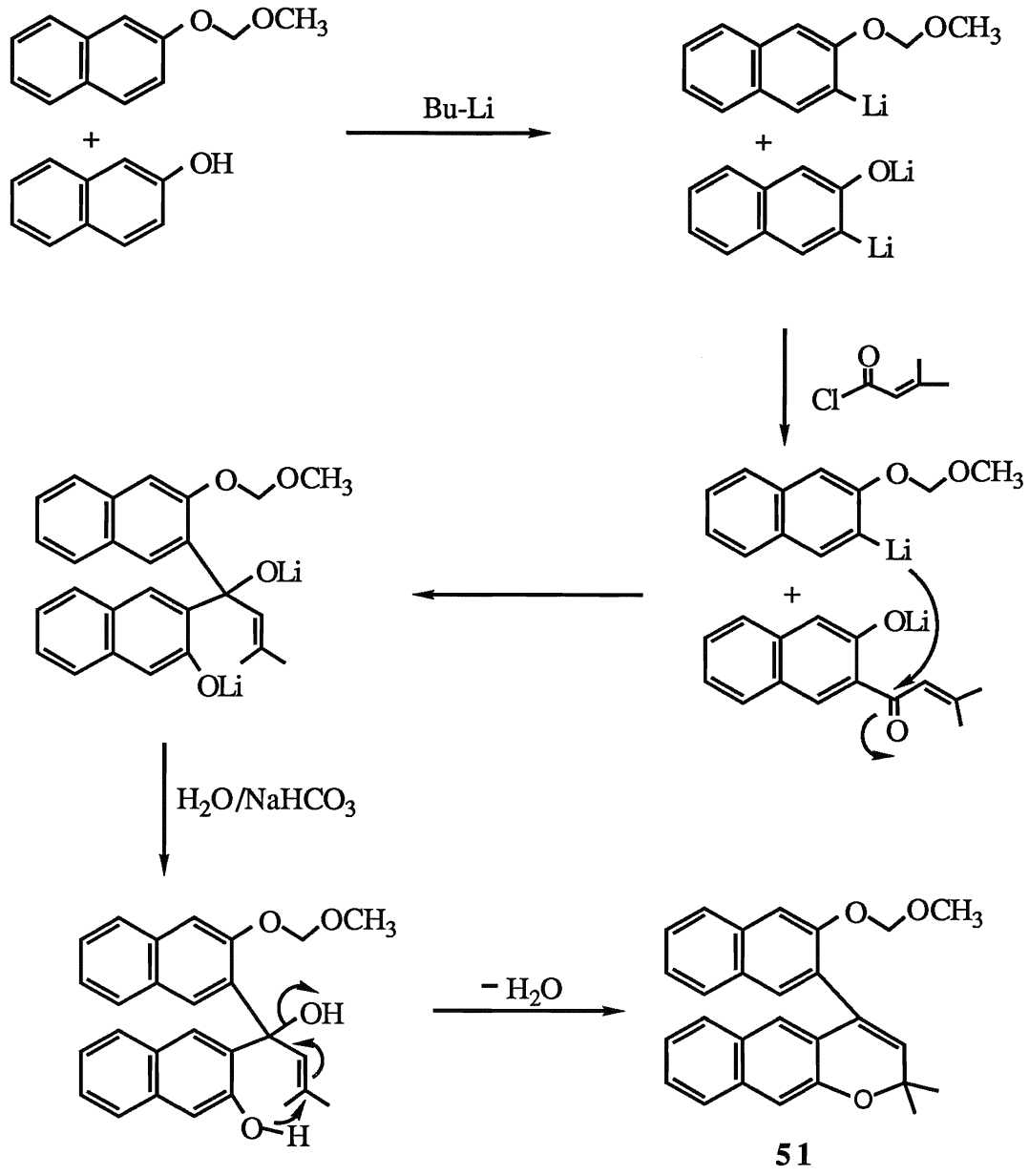
Scheme-37

Butyllithium was also used to test our suggestion. The lithiation was successful, but we were not able to separate the products. The only compound that has been isolated was **51**; its structure is suggested by spectral analysis. ^1H NMR shows that there were 12 AR-**H,** and 4 singlet peaks in the aromatic region, 1H (O**H**, br), 1H (C=C**H**, S), 6H (2C$\mathbf{H_3}$, s), 3H (OC$\mathbf{H_3}$, s), and 2H (OC$\mathbf{H_2}$O, s); ^{13}C NMR Spectra shows that there were totally 22 carbon atoms in the conjugated aromatic region, which consisted of 13 CH with 9 C; 4 other peaks show OCH_2O at 95.5ppm, OC at 76.1ppm, OCH_3 at 56.1ppm, and CH_3 at 28.0. Combining all of the information that suggested structure **51**. The mechanism of its formation is proposed in Scheme-38:



51

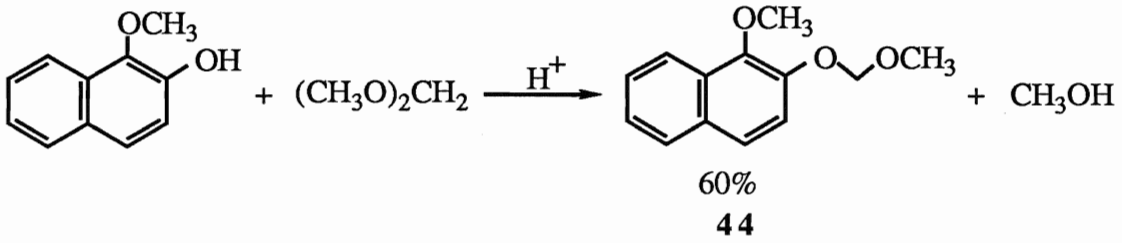
We believed that there was some unreacted 2-naphthol in the starting compound. The order of lithiation is: phenol OH, 3-H of the protected phenol, and 3-H of the phenol. The order of reaction with substrate is the reverse of the lithiation.



Scheme-38

Because dimethoxymethane has the advantages of being cheaper, easier to use, giving higher yields, and unreacted starting material can be easily recovered, we used dimethoxymethane as the reagent for the protection reaction. A similar reaction was done by Yardley, also with dimethoxymethane.[82] Therefore their procedure was used, but with a large excess dimethoxymethane. It has been found that the yield can be controlled by the recycle time of the solvent in the Soxhlet apparatus. The reaction gave a 60% yield of compound **44**.

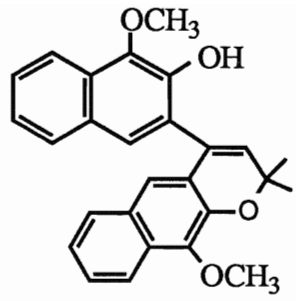
Equation-17:



60%
44

3. Lithiation of compound **44**

The lithiation of naphthalene and its derivatives (Scheme-39) has been well studied.[83,84] The relative rates of lithiation of 2-methoxynaphthalene was found to be 9.5:1.0 (C-3:C-1);[83] 1-methoxynaphthalene was 4.9:1.0 (C-2:C-8).[84]

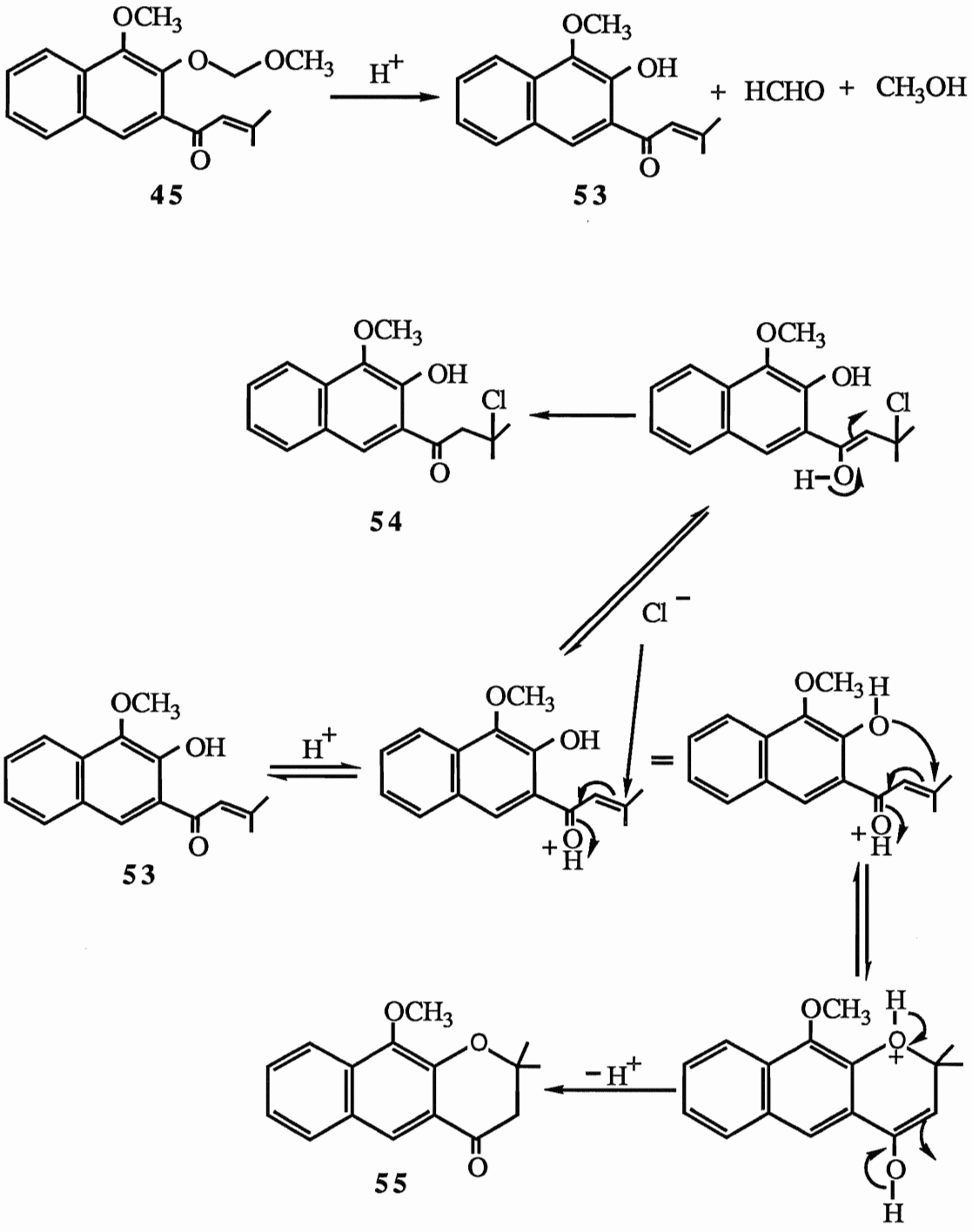


52

4. Preparation of compound **53** by hydrolysis of **45**

As we know, MOM ethers can be cleaved by acid (Equation-18). Recently, a study has been done by using diphosphorus tetraiodide cleavage of MOM ethers under mild conditions (short time). The mechanism of this reaction is not very clear. The procedure was used to give compound **53** in 75% yield. The MOM ether can also be removed very easily even by moisture (slightly acidic): There was a flask which containing **45**, which after two weeks changed from yellow to orange. ^{1}H NMR showed that compound **45** had been converted to compound **53** in 100% yield. 6N HCl was also used to remove the MOM ether, but three products were isolated: **53**, **54** and **55**. We believe that compounds **54** and **55** were formed from compound **53** (Scheme-41).

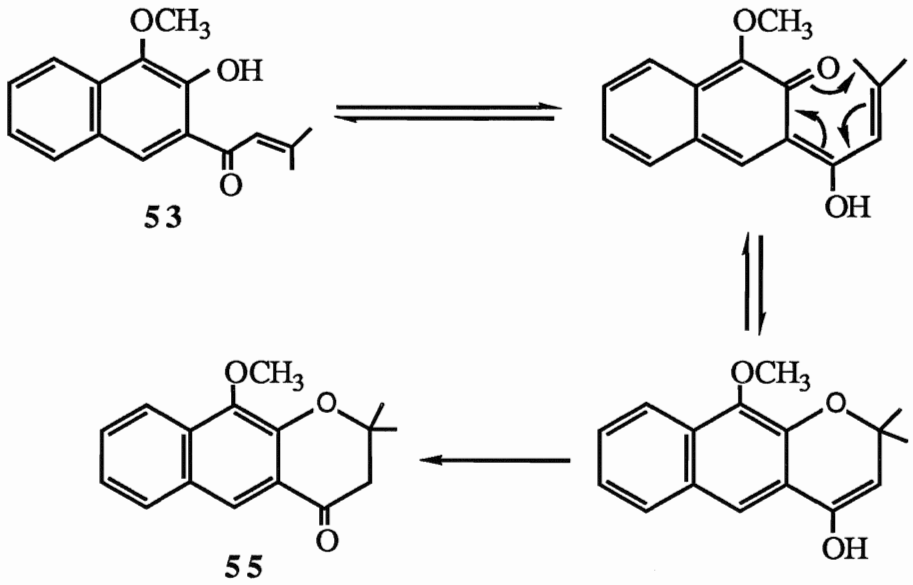
Equation-18:



Scheme-41

5. Cyclization of **53**

At first, HCl was used to give compound **55** by cyclization of **53**, but only in 50% yield and 50% was the starting compound **53**. This gave us some information about the mechanism of this reaction. We considered the mechanism as a Michael addition (Scheme-41). For this reason, a stronger Lewis acid (BF_3) was used as a catalyst for this reaction. The result was perfect, with 95.8% product. But this result was not repeatable; yields of repeat reactions were only around 10-20%. This reaction may also be a cycloaddition process (Scheme-42), and the product was formed in 20-30% (test by TLC) when **53** was refluxed in toluene. From these results it is not possible to say which mechanism is followed.



Scheme-42

6. One pot reaction: from **45** to **55**.

Finally, H_2SO_4 was used as a MOM cleavage reagent, because the HSO_4^- ion is not a strong nucleophile, unlike Cl^-. The yield of the reaction was very high, and up to 91% compound **55** was formed. Compound **45** was directly converted to **55** by refluxing in THF with 6N H_2SO_4.

IV. BIOTRANSFORMATION OF COMPOUND 55

As shown in Scheme-19, *Mortierella isabellina* ATCC 42613 produced *(S)*-alcohols from aryl ketones. 31% of 6,7-benzo-2,2-dimethyl-8-methoxy-4-chromanol **56** was produced from its ketone by *Mortierella isabellina* ATCC 42613. However the e.e.(enantiomeric excess) of this compound was only 33% as determined by chiral shift reagent study with tris[3-(heptafluoropropylhydroxymethylene)-(1R)-camphorato] europium(III) **8**. The proton on the chiral carbon was split as two peaks. The optical rotation shows positive, which is the same as the products of **27** and **28**. Therefore we assume our product has the same configuration *(S)* . The yield has been improved to 76% by controlling the age of the culture (Table-5), but the product was still not good enough for our requirements.

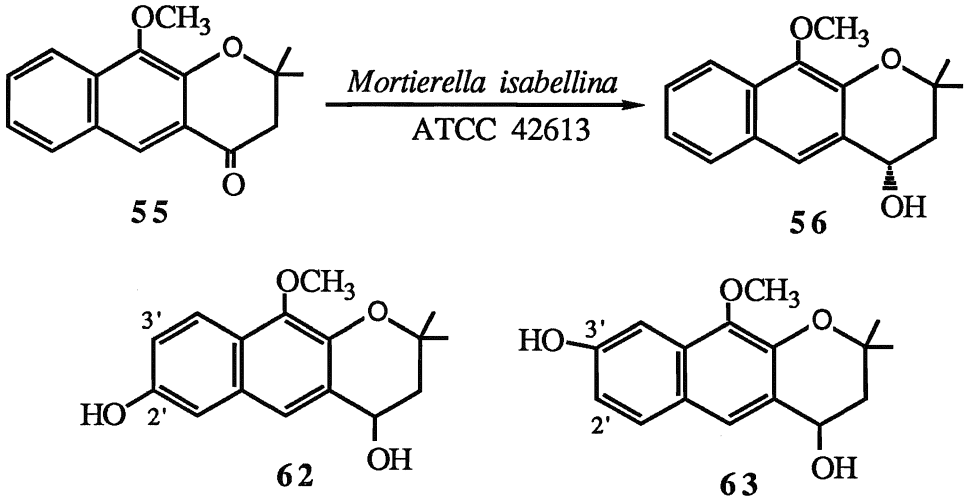
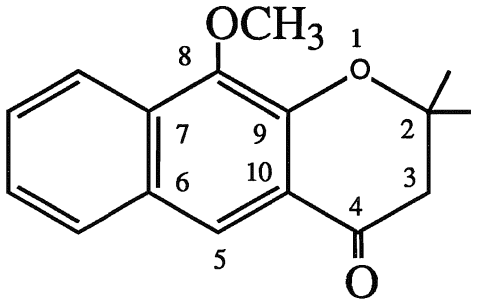


Table-5:

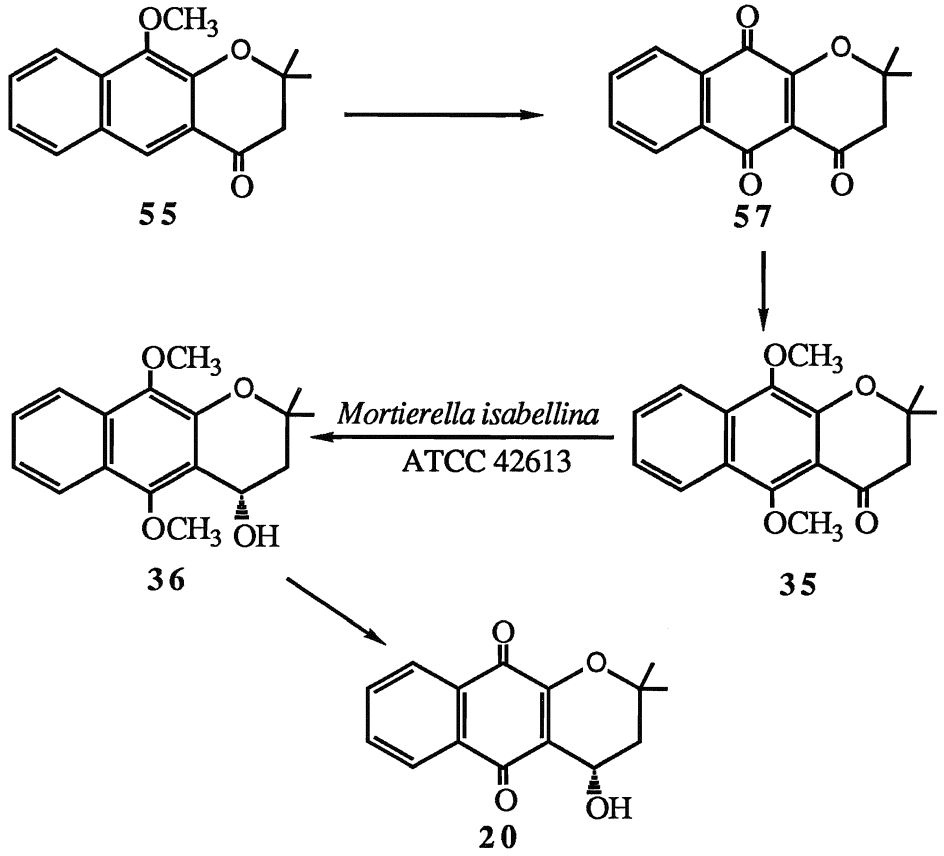
Age of culture	e.e.(%)
40 hrs	10
72 hrs	33
96 hrs	76
120 hrs	72

Another product was also isolated. ^{1}H NMR data showed two singlet aromatic H's and two AB coupled aromatic H's. Combined with mass data that suggested the structure of the unknown was either 6,7(2'-hydroxybenzo)-2,2-dimethyl-8-methoxy-4-chromanol **62** or 6,7(3'-hydroxybenzo)-2,2-dimethyl-8-methoxy-4-chromanol **63**.



55

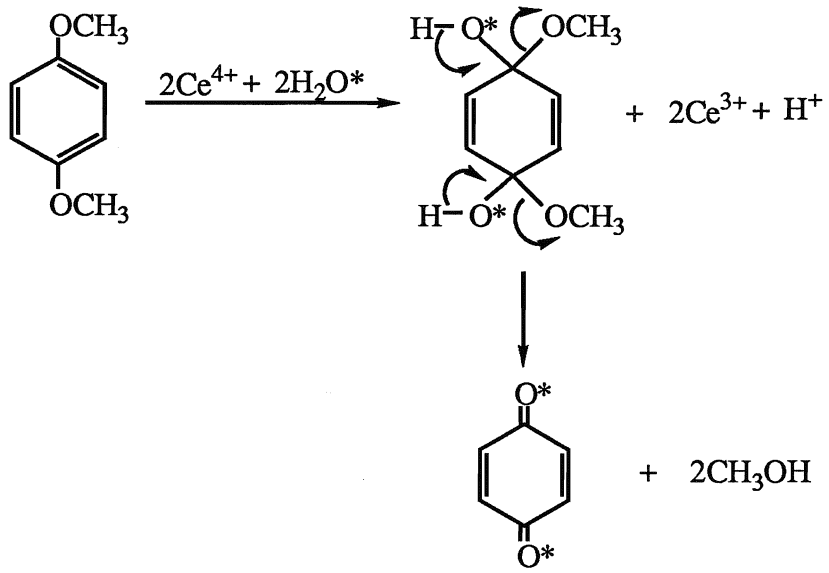
The reason for this lack of stereospecifity in reduction is that compound **31** may not be large enough to fully occupy the enzyme's active site. A compound which has a group or an atom at C-5 can be used to solve this problem. Scheme-43 was the suggested solution.



Scheme-43

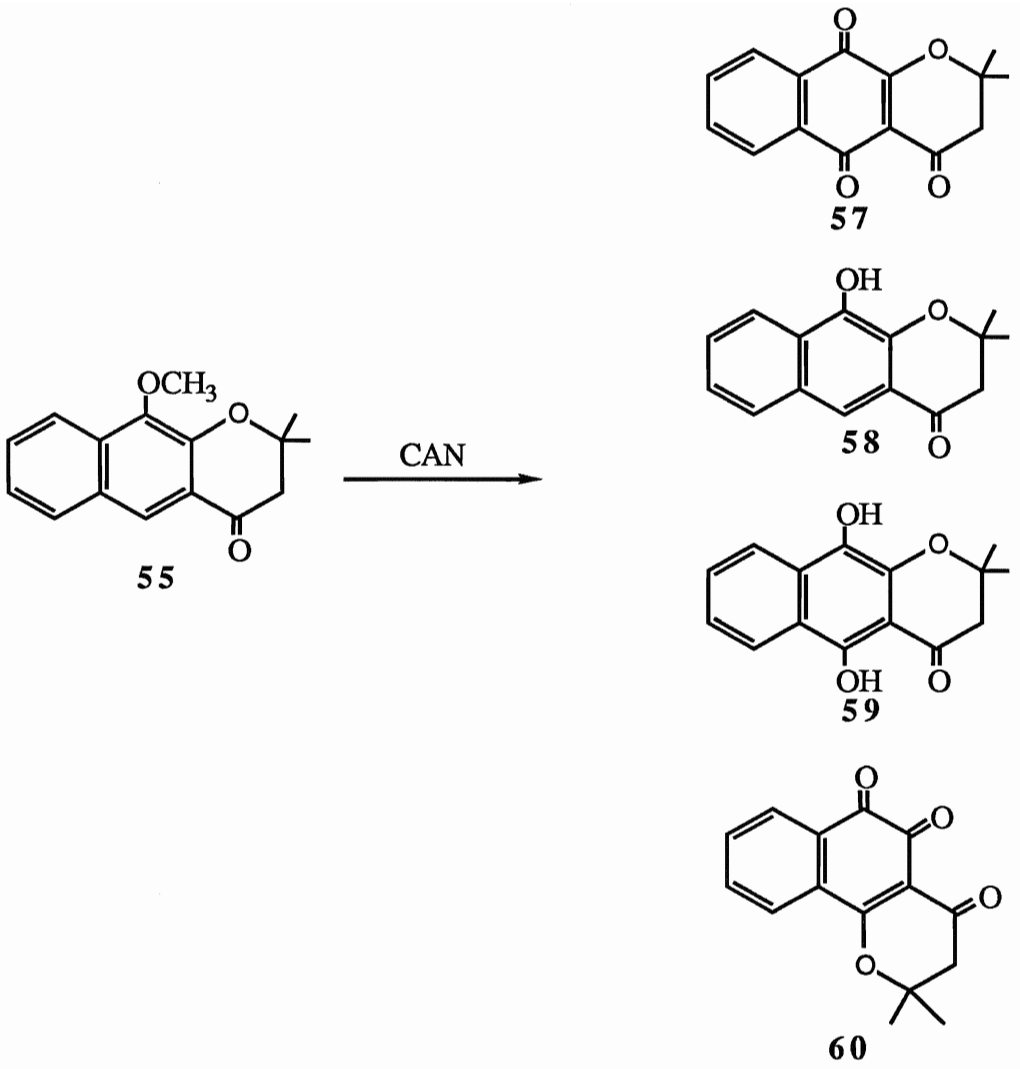
V. OXIDATION OF COMPOUND 55

There are many oxidation reagents which can be used to produce quinones. It has been known that hydroquinone dimethyl ethers can be oxidized to their corresponding quinones by using silver(II) dipicolinate ($(DPAH)_2Ag \cdot H_2O$) **5**,[15] or ceric ammonium nitrate (CAN).[16] The proposed mechanism is shown in Scheme-44:[16]



Scheme-44

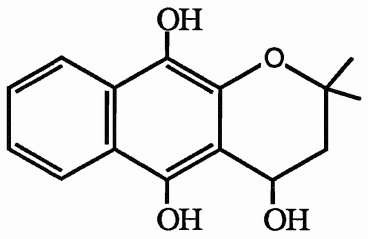
There are no reports for oxidation of monomethoxy ethers to quinone, but we assumed that should be a similar reaction as the oxidation of dimethoxy ether. Therefore, compound **55** was oxidised with CAN giving the relatively unstable monohydroxy compound **58** as the major product (Scheme-45). Further oxidation with air yielded **57** (53%), **58** (14%), **59** (4%), with trace amount of **60** whose structure is suggested from analytical data: mass=256 shows that it is a isomer of **57**; IR: 1767cm^{-1} (C=O), 1712cm^{-1} (C=O), 1698cm^{-1} (C=O); ^{1}H NMR shows 1.6 (6H,s,C**H**$_3$), 2.7 (2H,s,C**H**$_2$), 7.76 (2H,m,Ar**H**), 8.0 (1H,d,Ar**H**), 8.2 (1H,d,Ar**H**) which also confirmed the structure **60**.



Scheme-45

At this time, the steric effect of the skeleton of **55** had been increased by introducing an oxygen atom at C-5 (**57** and **59**). We suggested there would be four possible compounds found in the product of the biotransformation of **57**: a), unreacted starting material **57**; b), the enzyme may only reduce **57** to **59**; c), all of three carbonyl groups would be reduced to hydroxyl groups to give

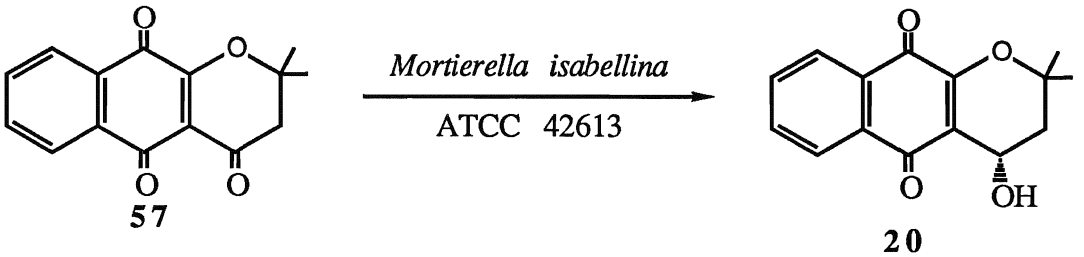
61; d), Compound **20** would be produced by regiospecific reduction. Therefore, it was necessary to find the result of the biotransformation of **57**.



61

VI. PREPARATION OF *(S)*-4-HYDROXY-α-LAPACHONE 20:

Enzymic reactions are not only stereo-specific, but regio-specific as well. This is evident for our reaction in reduction of **57**. The 4-carbonyl group of **57** has been reduced regio-specifically, and stereo-specifically to give the *(S)* alcohol **20** with 49% yield, and e.e.>98% which was determined by chiral shift reagent **8**. The state of 4-hydroxy-α-lapachone has been reported differently as an oil and a solid (see Table-7). Our product is a solid with m.p.=67°C.



57

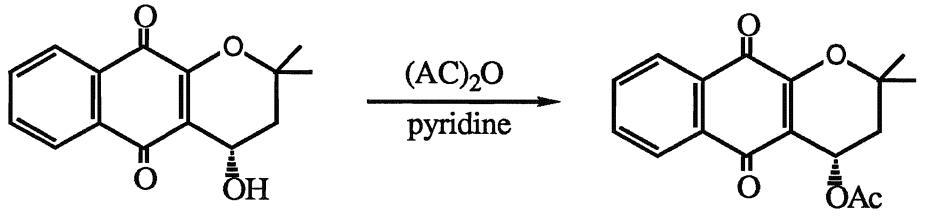
20

Yield 49%
e.e. >98%
M.p.=67°C

Scheme-46

VII. THE ACETYLATION OF COMPOUND 20

Although compound **20** is the natural product, not much analytical detail has been reported.[49,50,51,53,54] Most studies have been done on its acetyl derivative.[49,50,51,53,54] These reports included spectral and optical rotation data. The acetylation reaction was carried in dried pyridine which not only acts as a solvent, but also as a catalyst. The pure product **22** has a high melting point 145-148°C, higher than the reported values (136-140.5°C),[50,53,54] and an optical rotation $[\alpha]_{589}$ = -22.7 which is also higher than that reported $[\alpha]_{589}$ = -14.2.[50] The ^1H and IR spectra are, however, identical with those reported.[49,50,53,54] The presence of impurities would reduce both the melting point and the optical rotation, and so we believe our product to be purer than those earlier reported. Furthermore, our sample has identical UV peaks at 244.9, 250.4, 280.9 and 333.0 nm, but slightly lower ε values than these reported in the literature.[49,50,53]



20 **22**

Yield: 97%
M.P.:145-148.5°C

Scheme-37

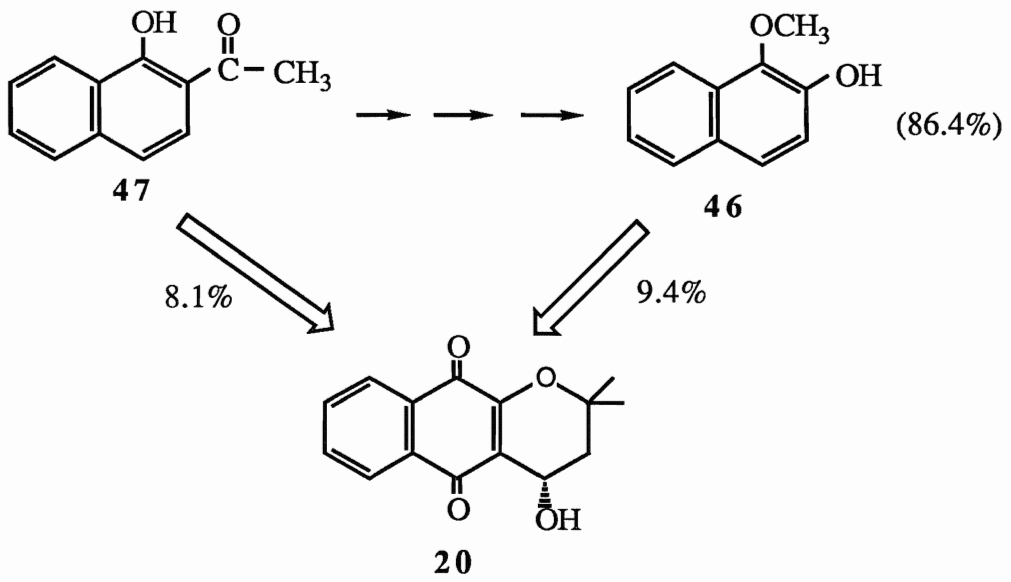
In the Table-6 shows the comparison ^1H NMR of compound **22**.

Table-6:

Ref.	C<u>H</u>3	C<u>H</u>3	COC<u>H</u>3	C<u>H</u>2	C<u>H</u>OAc	Ar<u>H</u>	Ar<u>H</u>
(49)	1.46(s)	1.50(s)	2.03(s)	2.1(d)	6.05(t)	7.5-8.1 (m)	
(50)	1.53(s)	1.57(s)	2.09(s)	2.17(d)	6.10(dd)	7.58-8.22(m)	
(54)	1.51(s)	1.56(s)	2.09(s)	2.16(ddd)	6.10(dd)	7.7(m)	8.1(m)
Our	1.51(s)	1.56(s)	2.15 (m)		6.10(dd)	7.72(m)	8.10(m)

SUMMARY:

(S)-4-hydroxy-α-lapachone **20** and *(S)*-4-acetoxy-α-lapachone **22** have.been synthesized in overall yields of 8% from 2-acetyl-1-naphthol **47**, and 9% from 1-methoxy-2-naphthol **46**. The chiral centre was introduced by reduction of the ketone **57** using *Mortierella isabellina* ATCC 42613.



Scheme-38

Finally Table-7 shows the physical data for **20** and **22**. From this comparison, we believe our product to be purer than others, and to be a single enantiomer *(S)*. The biological activity is under investigation.

Table-7:

Ref	20		22		Method	Chirality
	m.p.(°C)	$[\alpha]_D$	m.p.(°C)	$[\alpha]_D$		
(49)	yellow oil	N/L	118-120	N/L	Natural	*(S)*
(50)	yellow syrup	+27.4 (MeOH)	139.5-140.5	-14.2° (MeOH)	Natural	*(S)*
(53)	117-119	—	136-138	—	Synthetic	Racemic
(54)	yellow oil	—	138.5-139.5	—	Synthetic	Racemic
our product	67	+39.7 (EtOH)	145-148.5	-22.7 (MeOH)	Synthetic	*(S)*

EXPERIMENTAL

I. GENERAL

1. Spectrometers

IR spectra was done with an Analect FX 6260 FTIR spectrometer interfaced to an analect MAP-66 data system, or with a BOMEM FTIR (MB-Series). ^{1}H NMR spectra were recorded on a Bruker WP80CW, Bruker AC200 or Bruker AM500 spectrometer; ^{13}C NMR spectra on Bruker AC200 or Bruker AM500 spectrometer. Tetramethylsilane (δ=0) or chloroform-d (δ=7.25) were used as internal standards. A Kratos Concept 1S double focussing mass spectrometer operating in EI mode was used for the mass spectral analysis. UV spectra were recorded on a Varian DMS 100 UV Visible Spectrophotometer.

2. Chromatography

Thin Layer Chromatography (TLC) were done on E.Merck precoated (0.22 mm) Silica Gel 60 F_{254} plate. Column Chromatography was performed using Inter Science silica gel (mesh 70-230).

3. Melting Point

All melting points of products were measured on a Koffler hot stage apparatus.

4. The optical rotation were done on a RUDOLPH RESEARCH Autopol®III Automatic Polarimeter at ambient temperature.

5. Solvents

Commerical solvents were used for routine puposes. Ether, benzene and tetrahydrofuran (THF) were dried by distillation over sodium. CH_2Cl_2 was stored over molecular sieves (3Å or 4Å).

6. Chemicals

All of chemicals used for reactions were commercial material. Molecular sieves were dried at 300°C for least 3 hours before using.

n-Butyllithium and t-butyllithium were titrated with diphenyl-acetic acid.[85] 3,3-Dimethyl-acryloyl chloride (3-methyl-2-butenoyl-chloride) **18** was partially prepared from 3,3-dimethylacrylic acid **17**.[86]

II. THE FIRST APPROACH TO 6,7-BENZO-5,8-DIMETHOXY-2,2-DIMETHYL-2-CHROMANONE 35.

1. 1,4-Dihydroxynaphthalene **29.**

1,4-Naphthoquinone (30g, 0.19mol) was dissolved in 500mL of diether ether and sodium hydrosulfite ($Na_2S_2O_4 \cdot H_2O$) (60g,0.31mol) dissolved in 450mL of water was added and the mixture stirred vigorously until the color became pale brown (about 2 hrs). This mixture was transferred to a separatory funnel and shaken well. The inorganic layer was extracted by ether. Organic layers were combined together and washed with aq.satd. NaCl solution. Anhydrous

magnesium sulfate ($MgSO_4$) was used to dry the organic layer overnight. After removing the solvent, the dark-brown solid of crude 1,4-dihydroxynaphthalene **3** was obtained. The crude product was purified by column chromatogrphy on silica gel (chloroform) to give 16.71g (56%) of pure **29** as white needle crystals, m.p.207-208°C (Lit:[87] 195°C).

The structure of the compound **29** was evident by its spectral data which included: ^1H NMR ($CDCl_3$-DMSO) δ 6.70 (2H,s,Ar-H), 7.40 (2H,q,Ar-H), 8.15 (2H,q,Ar-H), 8.47 (2H,br,Ar-OH); MS (EI), m/z (%): 160 (M^+,100), 131 (36.8), 104 (18.9).

2. 1,4-Dimethoxynaphthalene **30**

1,4-Dihydroxynaphthalene **29** (15.13g, 0.095mol) was dissolved in 120mL 10% KOH solution and stirred for 30 mins. until the temperature dropped to room temperature. Dimethyl sulfate (21mL, 0.22mol) was added slowly. This mixture was stirred overnight at room temperature and then extracted with ether. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. The brown solid was obtained after removing ether. This crude product was purified on silica gel column using 50% hexane and 50% ethyl acetate as the eluant to get 15.80g (0.084mol) (88%) light yellow solid **30** with mp.: 85-86°C (Lit.:[88] 85-86°C). TLC. Rf=0.74 ($CHCl_3$); ^1H NMR δ: 3.94 (6H,s,-OC**H**$_3$), 6.70 (2H,s,Ar-**H**), 7.50 (2H,q,Ar-**H**), 8.20 (2H,q,Ar-**H**); MS (EI), m/z (%): 188 (M^+,79.6%), 173 (M^+-CH_3,100%),145 (14.5).

3. 1,4-Dimethoxy-2-naphthaldehyde **31**

1,4-Dimethoxynaphthalene **30** (8.6g, 0.046mol) was added to a mixture of 7.2mL (0.06mol) N-methylformanilide and 5.4mL (0.06mol) phosphorus oxychloride ($POCl_3$) under dry conditions, and stirring continued for 20mins. This mixture was then heated at 78°C in a refluxing benzene bath overnight. Water (100mL) was added into the cooled mixture and stirring continued for 1 hour. The mixture was then extracted with $CHCl_3$, and dried with $MgSO_4$. After removing $CHCl_3$, the crude green solid was purified on a silica gel column using $CHCl_3$ as a eluant giving 9.1g slight yellow solid mp: 120-121°C (Lit.:[65] 125°C). The long needle crystalline **31** was obtained by recrystallization with $CHCl_3$ and hexane. TLC, Rf=0.60 ($CHCl_3$); IR: 1679cm^{-1} (C=O); ^1H NMR δ 4.02 (3H,s,OC**H**$_3$), 4.10 (3H,s,OC**H**$_3$), 7.63 (2H,m,Ar**H**), 8.20 (2H,m,Ar**H**), 10.58 (1H,s,C**H**O); ^{13}C δ 55.6 (O**C**H_3), 65.8 (O**C**H_3), 98.4 (**C**H=C), 123.0 (**C**H=C), 124.7 (**C**=C), 127.3 (**C**H=C), 128.6 (**C**=C), 128.9 (**C**H=C), 130.4 (**C**=C), 152.3 (0**C**=C), 157.1 (O**C**=C), 189.6 (-**C**HO); MS (EI) m/z (%), 216 (M^+,100%), 201 (M^+-CH_3,68%), 187 (10%), 173 (30%), 145 (20%).

4. 1,4-Dimethoxy-2-naphthol **32**

A. Using m-Chloroperbenzoic acid

1,4-Dimethoxy-2-naphthaldehyde **31** (5.4g, 0.025mol) in 30mL CH_2Cl_2 was added slowly into an ice-cooled solution of m-chloroperbenzoic acid (10.3g, 0.04mol) in 30mL CH_2Cl_2 over a one hour period. This solution was stirred overnight at room

temperature. The color of the solution changed to yellow. 10% $NaHSO_3$ (100mL) was added then and stirring continued for another half hour. This time the color of the solution changed to brown. This mixture was neutralized with satd. sodium bicarbonate ($NaHCO_3$) solution, extracted with CH_2Cl_2 and dried with $MgSO_4$. After removal of CH_2Cl_2, 3.8g iron-red oil, which solidified overnight, was obtained. This crude product was dissolved in 15mL methanol. After 14mL 10% NaOH was added the color of the solution changed to brown. This solution was stirred overnight at room temperature, and acidified to pH=1 with conc. HCl, extracted with $CHCl_3$ and dried over $MgSO_4$. A yellow solid was obtained after removing $CHCl_3$, and purified on a silica gel column with $CHCl_3$ as the eluant, giving 2.31g (45%) of compound **32**. Spectral data for **32** are listed under b), below.

B. Using peracetic acid

Peracetic acid solution (6.2mL, 0.028mol of 36%-40% peracetic acid) was added into a solution of 0.1g anhydrous sodium acetate in 1.5mL acetic acid. Compound **31** (5.0g, 0.018mol) in 3mL acetic acid was added dropwise into the above peracetic acid solution under stirring conditions. This mixture was then stirred at 40-42°C for 20 hours, then at room temperature for 24 hours. When 10% $NaHSO_3$ solution was added, a brown oil was formed at the bottom of the flask. Ether was then added to bring the oil into solution. The solution was neutralized with $NaHCO_3$ until CO_2 evolution was completed. The organic layer was separated, the aqueous layer was

extracted with ether, and combined organic layers washed with brine. A brown oil was obtained after removing ether. Crude compound **32** (1.8g, 0.0088mol, 49%) was obtained from silica gel chromatography using hexane and ethyl acetate gradient solvents as eluant.

Compound **32**: mp: 92-94°C (Lit.:[67] 95°C); IR, 3394cm^{-1} (OH); MS (EI) m/z (%), 204 (M^+,53%), 189 (M^+-CH_3,100%), 175 (9%), 161 (70%), 105 (31%); ^{1}H NMR δ 3.91 (3H,s,OC**H**$_3$), 3.95 (3H,s,OC**H**$_3$), 5.95 (1h,br,O**H**), 6.58 (1H,s,Ar**H**), 7.31 (1H,t,CH=C**H**-CH), 7.49 (1H,t,CH=C**H**-CH), 7.82 (1H,d,C**H**=CH) 7.85 (1H,d,C**H**=CH); ^{13}C NMR δ 55.8 (O**C**H_3), 61.5 (O**C**H_3), 96.6 (**C**H=C), 119.9 (**C**H=C), 121.3 (**C**=C), 122.5 (**C**H=C), 122.7 (**C**H=C), 127.0 (**C**H=C), 128.2 (**C**=C), 133.1(**C**=C), 145.3 (**C**=C), 153.1 (**C**=C).

5. 1,4-Dimethoxy-2-naphthanyl tetrahydropyranyl ether **33**

2 Drops conc. HCl were added to a solution of compound **32** (0.50g,0.0025mol) in DHP (0.45mL 0.005mol). This solution was stirred overnight at room temperature. CH_2Cl_2 (30mL) was added, and this solution was washed with 10% NaOH twice and water once, and then dried over $MgSO_4$. A yellow oil was obtained after removal of CH_2Cl_2. 1.6g of crude yellow oil was isolated from a silica gel column and was identified by ^{1}H NMR as compound **33** with impurities. ^{1}H NMR δ 3.95 (3H,s,OC**H**$_3$), 3.96 (3H,s,OC**H**$_3$), 5.55 (1H,t,OC**H**CH_2), 6.60 (1H,s,Ar**H**), 7.35 (1H,t, CHC**H**=CH), 7.48 ((1H,t, CHC**H**=CH), 8.06 (1H,d,C**H**=CH), 8.15 (1H,d,C**H**=CH). Attempts to further purify **33** led to decomposition.

III. THE SECOND APPROACH TO 6,7-BENZO-5,8-DIMETHOXY-2,2-DIMETHYL-2-CHROMANONE 35.

1. Preparation of the silver salt of hydroxynaphthoquinone

2-Hydroxy-1,4-naphthoquinone (lawsone) **38** (5.0g, 0.028mol) was covered in 75mL hot water and enough ammonium hydroxide was added to bring it into solution. Dilute nitric acid was added to neutralize the slight excess of ammonia until formation of precipitate after prolonged stirring of the solution. The solution was then filtered and treated with the solution of silver nitrate (5.47g, 0.32mol) in 20mL water. The dark red salt was filtered and washed with water, ethanol and ether, then dried in a vacuum oven at 50°C overnight, to give **39** 7.65g red solid (96.6%).

2. 2-(Methoxymethoxy)-1,4-naphthoquinone **40**

To a suspension of 1.4g (0.004mol) of the silver salt of lawsone in 10mL of dried benzene, 0.45mL (20% excess) of chloromethyl methyl ether was added slowly at 0°C. As soon as the red color disappeared, the reaction was diluted with ether and filtered. The filtrate was washed twice with 6N NH_4OH, once with 1N NaOH, and finally with brine. After drying with $MgSO_4$ and removal of sovents, a yellow solid **40** was obtained. Yield: 0.369g (42%); M.p.:116-117°C (Lit.:[73] 116-118°C); ^1H NMR δ 3.54 (3H,s,OC<u>H</u>$_3$), 5.32 (2H,s,OC<u>H</u>$_2$O), 6.47 (1H,s,C<u>H</u>=C), 7.73 (2H,m,Ar<u>H</u>), 8.11 (2H,m,Ar<u>H</u>).

3. 1,4-Dimethoxy-2-(methoxymethoxy)-naphthalene **41**

To a solution of (1.78g, 8.17mmol) 2-(methoxymethoxy)-1,4-naphthoquinone **40** in 30mL acetone was added a solution of 7g of sodium dithionite in 30mL water. The mixture was stirred overnight at room temperature. After removing most of the acetone, a solution of 5.8g potassium hydroxide in 15mL water was added, followed by 12mL of dimethyl sulfate at 0°C. After a few minutes the ice bath was removed and stirring was continued at room temperature for 7 hours. The mixture was then extracted with chloroform, the organic layer washed with brine, and dried with $MgSO_4$. The crude product was obtained after removing solvent. 0.518g (26%) Light yellow oil compound **41** (Lit:[73] m.p.:33-34°C) was obtained from chromatography on silica gel with gradient 2% ether in hexane as eluant. Rf=0.47 (60%ether,40%hexane); NMR δ 3.56 (3H,s,OC<u>H</u>$_3$), 3.94 (3H,s,OC<u>H</u>$_3$), 3.95 (3H,s,OC<u>H</u>$_3$), 5.29 (2H,s,OC<u>H</u>$_2$O), 6.75 (1H,s,Ar<u>H</u>), 7.35 (1H,t,Ar<u>H</u>), 7.48 (1H,t,Ar<u>H</u>), 8.05 (1H,d,Ar<u>H</u>), 8.15 (1H,d,Ar<u>H</u>); MS (EI) m/z (%), 248 (M^+,61%), 218 (M^+-2CH_3,15.6%), 203 (M^+-3CH_3,82.4%), 175 (100%), 102 (32.5%).

4. 3-D-1,4-Dimethoxy-2-(methoxymethoxy)-naphthalene **37**

t-Butyllithium (4mL, 6.0mmol, 1.5M) was added slowly into a solution of compound **41** (0.5g, 2.0mmol) in 3mL ether (dried over Na) at 0°C, and the mixture was stirred for another 4 hours at this temperature. To this mixture, 2mL D_2O was then added. The mixture was diluted with ether and washed with sat.d sodium bicarbonate, brine and dried with $MgSO_4$. The crude product was obtained after

removing solvent. The NMR of the crude product **37** shown a 10% unreacted starting material when compared with the NMR of compound **41** (3-H changed to 3-D, 1H at δ=6.75 disappeared).

5. Lithiation of compound **41**

Compound **41** (0.55g, 2.2mmol) was dissolved in 5ml ether (dried over Na). t-Butyllithium (4ml, 6.0mmol,1.5M) was added into the above solution at 0°C and the mixture was continually stirred for 4 hours at this temperature. 3,3-Dimethylacryloyl chloride was then added into the mixture. The workup involved dilution with methylene chloride, washing twice with brine and drying with $MgSO_4$. After removing the solvent, the NMR of the crude residue showed unreacted starting compound **41** and mixture of unidentifiable products.

IV. THE THIRD APPROACH:

1. Preparation of 2-(methoxymethoxy)-naphthalene **50**:

A. Using chloromethylmethyl ether:

2-Naphthol (6.5g, 0.045mol) was dissolved in 30mL EtOH. 2.8g Potassium hydroxide was dissolved in the solution for half hour. Chloromethylmethyl ether (4.5mL, 0.58mol) was then added slowly to the ice cooled solution, and the mixture then stirred for a further 2 hours. Chloroform was used to extract the product, and the extract was washed with 10% NaOH, Satd. $NaHCO_3$, and dried with $MgSO_4$. After removal of solvents, the crude product was purified by

chromatography on silica gel using $CHCl_3$ as eluant to get 3.8g (45%) slight yellow oil **50**. Analytical data are shown below in b).

B. Using dimethoxymethane:

2-Naphthol (3.6g, 0.025mol), dimethoxymethane (10mL, 0.11mol), dichloromethane (70mL), and p-toluenesulfonic acid monohydrate (0.025g) were placed in a 100mL round-bottomed flask which was fitted with a Soxhlet apparatus containing 15g of 3Å molecular sieves. The reaction was refluxed overnight under Ar. After cooling the mixture, 0.2mL $(Et)_3N$ was added. The mixture was then washed twice with 10% sodium hydroxide to recover the unreactive starting material, once with brine and dried with $MgSO_4$. Compound **50** (2.5g, 55%) was obtained after chromatography on silica gel using $CHCl_3$ as eluant. ^1H NMR δ 3.49 (3H,s,C**H**$_3$), 5.62 (2H,s,OC**H**$_2$O), 7.65 (4H,m,Ar**H**), 7.98 (3H,m,Ar**H**); ^{13}C NMR δ 56.0 (**C**H_3), 94.5 (O**C**H_2O), 110.0 (**C**H), 118.9 (**C**H), 124.0 (**C**H), 126.3 (**C**H), 127.0 (**C**H), 127.6 (**C**H), 129.4 (**C**H), 129.5 (**C**), 134.5 (**C**), 154.8 (**C**),

2. Lithiation of **50**:

2-Methoxymethoxynaphthalene **50** (2.3g, 0.012mol) was dissolved in 50mL of dry THF and stirred under Ar. The mixture was cooled to 0°C and n-butyllithium (10mL, 0.02mol, 2M) was slowly added via a syringe. The reaction was exothermic and the temperature rose up to 5°C, The mixture was then stirred for 2 hours at room temperature. The mixture was then cooled to -65°C and 3,3-dimethylacryloyl chloride (2.1mL, 0.019mol) was added slowly. The mixture was then

stirred overnight at room temperature. Water was added to the reaction, and $CHCl_3$ was used to extract the product. The extract was washed with brine and dried over $MgSO_4$. After removal of solvent, chromatography was used to purify the product with $CHCl_3$ as eluant. Only compound **51** was isolated: ms: m/z (%), 396 (M^+, 32.8%), 381 (100%), 351 (23.9%), 335 (33.0%), 149 (59.9%); ^1H NMR δ 1.56 (6H,s,C**H**$_3$), 3.25 (3H,s,C**H**$_3$), 5.14 (1H,br,O**H**), 5.88 (1H,s,C=C**H**), 7.14 (1H,s,Ar**H**), 7.17 (1H,t,d,Ar**H**), 7.23 (1H,s,Ar**H**), 7.31 (1H,t,d,Ar**H**), 7.39 (1H,t,d,Ar**H**), 7.46 (2H,m,Ar**H**), 7.51 (1H,s,Ar**H**), 7.63 (1H,d,Ar**H**), 7.76 (1H,s,Ar**H**), 7.80 (2H,t,Ar**H**). ^{13}C NMR δ 28.0 (**C**H$_3$), 56.1 (O**C**H$_3$), 76.1 (O**C**), 94.5 (O**C**H$_2$O), 109.1 (**C**H), 111.5 (**C**H), 123.6 (**C**H), 124.0 (**C**), 124.4 (**C**H), 124.6 (**C**H), 126.1 (**C**H), 126.3 (**C**H), 126.5 (**C**H), 126.9 (**C**H), 127.6 (**C**H), 129.2 (**C**), 129.4 (**C**), 129.7 (**C**), 130.4 (**C**H), 132.3 (**C**), 133.0 (**C**H), 134.4 (**C**), 134.6 (**C**), 151.0 (**C**), 153.3 (**C**).

3. Preparation of 2-acetyl-1-methoxynaphthalene **48**:

To a hot solution of 2-acetyl-1-naphthol (20g, 0.108mol) in 100mL EtOH was added in five portions a solution of NaOH (5.0g) in 15mL H_2O and dimethyl sulfate (33g, 0.26mol). The color of the mixture changed from green to colorless at the end of 20 minutes. Extra sodium hydroxide 5g in 15mL H_2O was then added and the mixture then refluxed for 3 hours. Evaporation the most of the EtOH and extraction with chloroform gave an extract which was washed with brine and dried with $MgSO_4$. The crude product then was purified by chromatography on silica gel with 20% ethyl acetate and 80% hexane. Compound **48** was obtained as a white solid, Yield:

20.78g (96%); Rf: 0.35 (80% hexane, 20% ethyl acetate); M.p.:46-47°C (Lit:[89] 45-47°C); IR, 1666.cm^{-1} (C=O) MS (EI) m/z (%), 200 (M^+,68.2%), 185 (M^+-CH_3, 100%), 170 (M^+-$2CH_3$, 29.2%), 127 (22.7%), 114 (29.3%); ^{1}H NMR δ 2.72 (3H,s,C**H**$_3$), 3.91 (3H,s,OC**H**$_3$), 7.52 (3H,m,Ar**H**), 7.72 (1H,d,Ar**H**), 7.77 (2H,m,Ar**H**), 8.15 (2H,m,Ar**H**); ^{13}C NMR δ 30.4 (**C**H$_3$), 63.4 (O**C**H$_3$), 123.1 (**C**H), 123.8 (**C**H), 125.2 (**C**H), 126.3 (**C**H), 127.5 (**C**), 127.7 (**C**H), 127.9 (**C**H), 136.6 (**C**), 157.2 (**C**), 199.4 (**C**=O).

4. Preparation of 1-methoxy-2-naphthyl acetate **49**:

A mixture of 41g (32%) peracetic acid and 3.2g sodium acetate in 160mL acetic acid was added to a solution of compound **48** (20g, 0.1mol) in 24mL acetic acid over a one hour period at 40°C. After 14 hours stirring at 40°C, the color of the solution had changed from light green to light yellow. The mixture then was cooled, treated with excess of aqueous sodium bisulfite, and extracted with chloroform. The organic layer was dried with $MgSO_4$. The crude light yellow solid **49** was obtained after removing the solvent. Yield: 21.56g (90%); Crude m.p. 85-88°C (lit.:[81] 92-93°C); Rf: 0.34 (80% hexane, 20% ethyl acetate); IR 1757cm^{-1} (OC=O); MS (EI) m/z (%), 216 (M^+,28.1%), 174 (100%), 159 (88%), 145 (7.0%), 131 (18.3%); ^{1}H NMR δ 2.39 (3H,s,C**H**$_3$), 3.97 (3H,s, -OC**H**$_3$), 7.19 (1H,d,Ar**H**), 7.48 (2H,m,Ar**H**), 7.49 (1H,d,Ar**H**), 7.81 (1H,dd,Ar**H**), 8.13 (1H,dd,Ar**H**); ^{13}C NMR δ 20.6 (**C**H$_3$), 61.6 (O**C**H$_3$), 122.1 (**C**H), 122.1 (**C**H), 124.2 (**C**H), 125.9 (**C**H), 126.2 (**C**H), 127.9 (**C**H), 128.8 (**C**), 132.7 (**C**), 139 (**C**), 146.4 (**C**), 169.1 (**C**=O);

5. Preparation of 1-methoxy-2-naphthol **46** :

Compound **49** (19.62g, 0.0908mol) was dissolved in a solution of 20mL conc. H_2SO_4 and 1200mL methanol. The mixture was then stirred for 20 hours at room temperature. At the end of the reaction, the mixture was poured into water, extracted with chloroform and dried with $MgSO_4$. The crude slight yellow solid product **46** was obtained after removing the solvent. Yield: 17.93g (100%); m.p.90-92.5°C (Lit.:[81] 92.5-93°C); IR: 3412cm^{-1} (-OH); ^{1}H NMR δ 3.96 (3H,s,OC**H**$_3$), 5.86 (1H,b,O**H**), 7.22 (1H,d,Ar**H**), 7.33 (1H,t,Ar**H**), 7.51 (2H,m,Ar**H**), 7.78 (1H,d,Ar**H**), 7.94 (1H,d,Ar**H**); ^{13}C δ 61.6 (O**C**H$_3$), 117.5 (**C**H), 120.3 (**C**H), 123.5 (**C**H), 125.3 (**C**H), 126.3 (**C**H), 128.0 (**C**), 128.3 (**C**H), 129.6 (**C**), 139.3 (**C**), 145.4 (**C**);

6. Preparation of 1-methoxy-2-(methoxymethoxy)-naphthalene **44**:

Compound **46** (6.26g, 0.036mol), dimethoxymethane (25mL, 0.28mol), dichloromethane (80mL), and p-toluenesulfonic acid monohydrate (0.1g) were placed in a 200mL round-bottomed flask which was fitted with a Soxhlet apparatus containing 40g of 3Å molecular sieves. The reaction was refluxed overnight under Ar. After cooling the mixture, 1mL $(Et)_3N$ was added. The mixture was then washed twice with 10% sodium hydroxide to recover the starting compound **46**, once with brine and dried with $MgSO_4$. The crude product was purified on silica gel with 2% acetate in hexane. A slight yellow oil, compound **44**, was obtained. Rf=0.41 (20% ethyl acetate, 80% hexane); yield: 4.85g (62%); mass: found 218.0941,

Calc. $C_{13}H_{14}O_3$=218.0943; ^{1}H NMR δ 3.53 (3H,s,OC**H**$_3$), 3.99 (3H,s,OC**H**$_3$), 5.27 (3H,s,OC**H**$_2$O), 7.4 (4H,m,Ar**H**), 7.74 (1H,d,Ar**H**), 8.11 (1H,d,Ar**H**); ^{13}C NMR δ 58.2 (O**C**H$_3$), 61.1 (O**C**H$_3$), 96.0 (O**C**H$_2$O), 116.7 (**C**H), 121.4 (**C**H), 124.1 (**C**H), 124.5 (**C**H), 126.0 (**C**H), 127.6 (**C**H), 129 (**C**), 130.6 (**C**), 144 (**C**), 145 (**C**); MS (EI) m/z(%), 218 (M^+, 100%), 188 (M^+-OCH_2, 23.7%), 173 (71.5%), 145 (36.7%), 127 (33.1%).

7. Preparation of 3-(3'3'-dimethyl-acryloyl)-1-methoxy-2-(methoxymethoxy)-naphthalene **45**:

t-Butyllithium (8.2ml, 0.011mol, 1.4M) was added slowly to a solution of compound **5** (2.0g, 0.0092mol) in 30mL THF at -80°C. The color of the solution changed from light yellow to deep green. The mixture was allowed to warm up to room temperature by itself and stirred for another hour. At the end of the time, the mixture was cooled to -80°C and 3,3-dimethylacryloyl chloride (1.4ml, 0.0122mol) slowly added. The color of the mixture changed from green to light yellow. The reaction was stirred over night at room temperature. The work-up process involved adding extra THF and extracting with $CHCl_3$. The organic layer was washed with 10% NaOH, saturated sodium bicarbonate, brine, and dried with $MgSO_4$. Chromatography was used to purify the product. The compound **45**: light yellow oil; Rf=0.41 (30% ethyl acetate, 70% hexane); yield: 1.66g (60%); ^{1}H NMR δ 2.01 (3H,d,C**H**$_3$), 2.26 (3H,d,C**H**$_3$), 3.52 (3H,s,OC**H**$_3$), 4.04 (3H,s,OC**H**$_3$), 5.18 (2H,s,OC**H**$_2$O), 6.68 (1H,t,C**H**=C(CH)$_3$), 7.51 (2H,m,Ar**H**), 7.80 (2H,m,Ar**H**), 8.12 (1H,d,Ar**H**); mass: found 300.1386, Calc. $C_{18}H_{20}O_3$=300.1362; MS (EI) m/z(%), 300

(M^+, 21.0%), 285 (M^+-CH_3, 9.1%), 256 (25.7%), 200(33.9%), 83 ($CHCl_3$-Cl, 100%).

8. Preparation of 3-(3',3'-dimethylacryloyl)-1-methoxy-2-naphthol **53**:

A. Using P_2I_4:

Diphosphorus tetraiodide (1.0g) was added to a solution of compound **45** (0.71g, 0.00237mol) in 30mL CH_2Cl_2 (dried over molecular sieves) at 0°C. The mixture then was stirred at this temperature for 30 minutes and at 10°C for 10 minutes. At the end of the reaction, the mixture was directly charged on the top of a short silica gel column and eluted with ether to afford a crude product. Crude yield: 0.45g (75%). This crude product was checked by ^1H NMR spectra that showed a high percentage of the desired product. A silica gel column was used to purify the crude product. Spectral data for **53** are listed in c) below.

B. Using ambient moisture:

Compound **45** (1.23g,0.0041mol) was kept in a flask from a column. After 2 weeks, the color of the compound was changed from light yellow to deep orange. The ^1H NMR showed that 100% **45** had been converted to compound **53**.

C. Using hydrochloric acid

Compound **45** (0.56g, 0.00187mol)) was dissolved in 50mL THF and 50mL 6N HCl. The mixture was stirred overnight at room

temperature. The reaction was checked by TLC on crude product. It showed that all of the protecting group had been removed, but several spots showed the reaction was not as clean as using P_2I_4. Three compounds have been found after purifying.

Compound **53** 0.181g (38%): Rf=0.62 (17% ether, 83% benzene); Orange solid, m.p.: 58-61°C (recrystallized from benzene); Mass for $C_{16}H_{16}O_3$: found 256.1120, calc. 256.1099; IR:1642cm^{-1} (C=O); ^{1}H NMR δ 2.11 (3H,s,C**H**$_3$), 2.26 (3H,s,C**H**$_3$), 4.05 (3H,s,OC**H**$_3$), 6.97 (1H,s,C**H**=C), 7.34 (1H,t,Ar**H**), 7.55(1H,t,Ar**H**), 7.81 (1H,d,Ar**H**), 8.03 (1H,d,Ar**H**), 8.18 (1H,s,Ar**H**); ^{13}C NMR δ 21.5 (**C**H$_3$), 28.3 (**C**H$_3$), 60.6 (O**C**H$_3$), 120.2 (**C**H), 121.0 (**C**H), 123.1 (**C**), 124.0 (**C**H), 126.6 (**C**), 127.0 (**C**H), 128.9 (**C**H), 129.3 (**C**H), 132.1 (**C**), 141.4 (**C**), 149.4 (**C**), 158.8 (**C**), 196.6 (**C**=O); MS (EI) m/z(%), 256 (M$^+$, 100%), 241 (M$^+$-CH$_3$, 48%), 223 (25.5%), 201(67.0%), 200 (66.0%), 185 (45%), 157 (86%).

Compound **54**, 3-(3'-chloro-3'-methyl-butyl)-1methoxy-2-naphthol (0.349g, 63%): dark brown thick oil; IR: 1643cm^{-1} (C=O), 3440cm^{-1} (OH); ^{1}H NMR δ 1.42 (6H,s,C**H**$_3$), 3.37 (2H,s,C**H**$_2$), 4.04 (6H,s,OC**H**$_3$), 7.34 (1H,t,Ar**H**), 7.57 (1H,t,Ar**H**), 7.82 (1H,d,Ar**H**), 8.08 (1H,d,Ar**H**), 8.18 (1H,s,Ar**H**).

Compound **55**, 0.144g (30%). Spectral data for **55** are listed later below.

Trace amount of compound **52** has been found in one of reactions: Yellow oil; IR: 3423cm-1 (OH); ^{1}H NMR δ 1.66 (6H,s,C**H**$_3$), 4.04 (3H,s,OC**H**$_3$), 4.09 (3H,s,OC**H**$_3$), 5.8 (1H,br,O**H**), 6.00 (1H,s,C=C**H**), 7.05

(1H,s,Ar**H**), 7.24 (1H,m,Ar**H**), 7.45 (5H,m,Ar**H**), 7.82 (1H,d,Ar**H**), 7.08 (2H,m,Ar**H**); ^{13}C NMR δ 27.8 (**C**H_3), 61.0 (O**C**H_3), 61.5 (O**C**H_3), 76.1 (**C**), 77.3 (**C**), 119.8 (**C**H), 120.4 (**C**H), 120.7 (**C**H), 121.0 (**C**H), 124.0 (**C**H), 125.7 (**C**H), 126.0 (**C**H), 126.4 (**C**H), 127.5 (**C**), 127.8 (**C**), 127.9 (**C**H), 128.2 (**C**H), 128.9 (**C**), 129.0 (**C**), 129.3 (**C**), 131.0 (**C**), 133.8 (**C**H), 139.9 (**C**), 141.7 (**C**), 141.8 (**C**), 143.8 (**C**); MS (EI) m/z(%), 412 (M^+, 73.1%), 397 (M^+-CH_3, 100%), 382 (9.0%), 367 (20.4%), 176 (8.5%).

9. Preparation of 6,7-benzo-2,2-dimethyl-8-methoxy-4-chromanone **55**:

A. Using p-toluenesulfonic acid:

0.10g Compound **53** was dissoved in 10mL toluene. 0.05g p-Toluenesulfonic acid was added and the mixture was refluxed overnight. TLC showed that no reaction had occured.

B. Using 6N HCl:

(1), 30mL 6N Hydrochloric acid was added to the solution of 0.316g compound **53** in 14mL dichloromethane. The mixture was stirred overnight at room temperature. TLC showed that 50% product had been formed.

(2), 30mL 6N HCl was added to a solution of 0.68g compound **53** in 30mL dichloromethane. This was stirred overnight at room temperature. The mixture was extracted with chloroform, washed with sat.d sodium bicarbonate and brine, finally dried with $MgSO_4$.

The crude product was purified on a silica gel column with gradient 2% ethyl acetate in hexane. The slight yellow crystal **55** was obtained. yield: 0.41g (60%); m.p.: 94-95.5°C; Mass: found 256.1119, calc. for $C_{16}H_{16}O_3$ 256.1099; elemental analysis: found C,74.61% H,6.60%; calc. for $C_{16}H_{16}O_3$ C,74.98% H,6.29%; ^{1}H NMR δ 1.55 (6H,s,C**H**$_3$), 2.85 (2H,s,C**H**$_2$), 4.03 (3H,s,OC**H**$_3$), 7.36 (1H,t,Ar**H**), 7.54 (1H,t,Ar**H**), 7.67 (1H,d,Ar**H**), 8.08 (1H,d,Ar**H**), 8.28 (1H,s,Ar**H**); ^{13}C NMR δ 27.0 (**C**H$_3$),49.7 (**C**H$_2$), 61.0 (O**C**H$_3$), 70.0 (O**C**), 121.3 (**C**H), 122.0 (**C**), 123.0 (**C**H), 124.7 (**C**H), 126.1 (**C**), 126.6 (**C**H), 130.0 (**C**H), 132.6 (**C**), 142.5 (**C**), 146.2 (**C**), 193.0 (**C**=O); MS (EI) m/z(%), 256 (M^+, 100%), 241 (M^+-CH_3, 16%), 201 (49%), 200 (64%) 185 (25%), 157 (44%).

C. Using BF_3:

$BF_3.O(C_2H_5)_2$ (0.01mL, 0.0105g) was slowly added to a solution of **53** (0.019g, 0.072mmol) in 2mL dry ether at 0°C under Ar, and stirring continuted for 1 more hour at this temperature. The mixture was then stirred over night at room temperature, and evaporated the next morning. Water was added to the brown oily mixture, and extrated by ether. The organic layer was washed with saturated sodium bicarbonate, water and brine. After drying with $MgSO_4$, TLC showed only a single spot. Compound **9** 0.0182g (95.8%) was obtained. The procedure was repeated, but yields were never as good.

10. One pot reaction: preparation of compound **55** from compound **45**.

The mixture of (4.32g, 0.0144mol) compound **45** in 90ml THF and 10ml 6M H_2SO_4 was refluxed over night. 5% Sodium hydroxide was used to neutralize the mixture, and this mixture was extracted with chloroform, washed with saturated sodium bicarbonate, and dried with $MgSO_4$. Chromatography was then used to separate the mixture, and gave compound **53**: yield 0.183g (5%); Compound **55**: yield 3.35g (91%).

V. BIOTRANSFORMATION OF 6,7-BENZO-2,2-DIMETHYL-8-METHOXY-4-CHROMANONE 55 TO A CHIRAL ALCOHOL 56:

1. General Method:

The liquid growth medium of *Mortierella isabellina* ATCC 42613 was prepared from 40g glucose, 5g yeast extract, 5g sodium chloride, 5g potassium phosphate (dibasic), 5g soya flower, and 1L distilled water; 200mL of this medium were placed in each of 5 1L flasks, and the flasks then sterilized by autoclaving, inoculated, grown at 27°C for 3 days. After growing the fungi in this medium, the fungi were filtered, washed with distilled water and resuspended in distilled water spread over the same number of flasks as used for growing the fungi. Substrate 60mg in 2mL 95% EtOH was added to each flask, and incubation carried out for the specified time at 27°C. The fungi were filtered and the filtrate was continuously extracted with dichloromethane for three days.

The enantiomeric excesses were determined by ^1H NMR analysis using the chiral shift reagent, tris-[3-(heptafluoropropyl-hydroxymethylene)-(1R)-camphorato] europium(III) **8** in $CDCl_3$-TMS.

A solution of compound **55** (180mg, 0.7mmol) in 6mL 95% ethanol was injected into 3 flasks which contained 4 days old culture. After 3 days bioconversion, the work up was performed as described above. The yellow oil obtained by CH_2Cl_2 extraction, was chromatographed on a silica gel column with gradient 2% ether in benzene as eluant. 35mg (20%) 6,7-Benzo-2,2-dimethyl-8-methoxy-4-chromanol **56** was isolated as a yellow oil. e.e.: 31% (from ^1H NMR); $[\alpha]_D$= +34.8 (c=0.49, EtOH); Mass: found 258.12703, calc. for $C_{16}H_{18}O_3$ 258.1256; MS (EI) m/z(%), 258 (M^+, 45.8%), 240 (M^+-OH_2, 60.3%), 225 (100%), 210 (29.9%) 202 (81.4%), 187 (55.3%); IR 3400cm^{-1} (OH); ^1H NMR δ 1.36 (3H,s,C**H**$_3$), 1.53 (3H,s,C**H**$_3$), 1.92 (1H,d,d,C**H**H-CH), 2.24 (1H,d,d,C**H**H-CH), 2.4 (1H,br,O**H**), 3.95 (3H,s,OC**H**$_3$), 5.02 (1H,t,C**H**-CH_2), 7.36 (2H,m,Ar**H**), 7.69 (1H,d,Ar**H**), 7.73 (1H,s,Ar**H**), 8.04 (1H,d,Ar**H**); ^{13}C NMR δ 25.9 (**C**H_3), 29.4 (**C**H_3), 42.8 (**C**H_2), 60.7 (**C**HOH), 64.1 (O**C**H_3), 75.8 (O**C**), 120.9 (**C**H), 121.5 (**C**H), 123.7 (**C**H), 125.9(**C**H), 127.6 (**C**H), 128.0 (**C**), 128.4 (**C**), 128.6 (**C**), 141.4 (**C**), 142.8 (**C**).

Compound **62** or **63**: 11mg green oil, IR: 3368cm^{-1} (OH); MS (EI) m/z(%), 274 (M^+, 2.1%), 256 (9.9%), 241 (9.4%), 226 (4.1%), 83 ($CHCl_2$, 100%); ^1H NMR δ 1.33 (3H,s,C**H**$_3$), 1.51 (3H,s,C**H**$_3$), 1.9 (1H,d,d,C**H**H-CH), 2.22 (1H,d,d,C**H**H-CH), 3.92 (3H,s,OC**H**$_3$), 4.95

(1H,t,C**H**-CH_2), 6.31 (1H,br,O**H**), 6.94 (1H,s,Ar**H**), 7.03 (1H,d,Ar**H**), 7.41 (1H,s,Ar**H**), 7.88 (1H,d,Ar**H**).

2. Age of culture and effect of pH:

180mg Compound **55** in 95% EtOH was injected into 3 1L flasks; one buffered at pH 4.5 by potassium hydrogen phthalate, one buffered at pH 7.5 by potassium dihydrogen phosphate and one with distilled water, not buffered. Each contained a 40 hours old culture (*Mortierella isabellina* ATCC 42613) which was prepared as above. After incubation for 15 hours, the crude products were obtained by extraction. Chromatography as above gave the alcohol **56**. The enantiomeric excesses of **56** of these 3 flasks were all same (e.e.:10%) as determined by chiral shift reagent **8**.

The procedure was repeated with 72, 96 and 120 hour old cultures, obtaining e.e.s of 33%, 76% and 72%, respectively. In no case did the pH have any effect on the e.e..

VI. OXIDATION OF 55 BY CERIC AMMONIUM NITRATE (CAN):

1. Compound **55** (0.202g, 0.783mmol) was dissolved in 2mL acetonitrile and the solution cooled in a ice bath. A solution of CAN (2.18g, 3.9mmol) in 10mL water was slowly added to the solution of **55** at 0°C over 5 minutes. The color of the mixture changed from light yellow to deep orange. The mixture was stirred for a further 30 minutes at 0°C. At the end of this period, 20mL water was added to the mixture. Chloroform was used to extract the product from aqueous solution, and the extract was washed with brine and dried

with $MgSO_4$. Chromatography on silica gel was used to isolate products, using an ether benzene gradient as eluant. The following were obtained.

6,7-Benzo-8-hydroxy-2,2-dimethyl-4-chromanone **58** 97mg (51%): yellow solid, m.p.:124°C; Rf=0.53 (ether); Mass: found 242.0927, calc. for $C_{15}H_{14}O_3$ 242.0943; ms: m/z (%), 242 (M^+, 46.2%), 223 (6.7%), 186 (100%), 158 (19.0%), 130 (26.0%), 102 (30.5%); ^1H NMR δ 1.53 (6H,s,C**H**$_3$), 2.86 (2H,s,C**H**$_2$), 5.93 (1H,br,O**H**), 7.37 (1H,t,Ar**H**), 7.52 (1H,t,Ar**H**), 7.85 (1H,d,Ar**H**), 8.06 (1H,s,Ar**H**), 8.11 (1H,d,Ar**H**); ^{13}C NMR δ 27.1 (**C**H_3), 50.0 (**C**H_2), 80.1 (**C**), 118.4 (**C**H), 120.63 (**C**), 121.1 (**C**H), 124.9 (**C**H), 127.3 (**C**), 127.8 (**C**H), 128.1 (**C**), 129.5 (**C**H), 139.3 (**C**), 192.8 (**C**=O).

6,7-Benzo-5,8-dihydroxy-2,2-dimethyl-4-chromanone **59**: yellow film; 11mg (5%); Mass: found 258.0880, calc. for $C_{15}H_{14}O_4$ 258.0892; ms: m/z (%), 258 (M^+, 83.8%), 243 (M^+-CH_3, 38.5%), 202 (100%), 174 (40.8%), 146 (58.7%), 105 (58.0%); ^1H NMR δ 1.51 (6H,s,C**H**$_3$), 2.82 (2H,s,C**H**$_2$), 5.34 (2H,br,O**H**), 7.37 (1H,t,Ar**H**), 7.58 (1H,t,Ar**H**), 8.03 (1H,s,Ar**H**), 8.28 (1H,d,Ar**H**).

4-Keto-α-lapachone **57**: 53mg (26%), yellow solid mp=145°C (Lit.:[50] 163-165°C); Rf: 0.17 (ether); IR: 1714cm^{-1} (C=O), 1689cm^{-1} (C=O); Mass for $C_{15}H_{12}O_4$: found 256.0751, calc. 256.0737; ms: m/z (%), 256 (M^+, 72.9%), 241 (M^+-CH_3, 21.5%), 201 (98.8%), 173 (47.2%), 104 (100%); ^1H NMR δ 1.62 (6H,s,C**H**$_3$), 2.77 (2H,s,C**H**$_2$), 7.76 (2H,m,Ar**H**), 8.10 (2H,m,Ar**H**); ^{13}C NMR δ 26.1 (**C**H_3), 48.7 (**C**H_2), 84.4

(**C**), 112.9 (**C**), 126.3 (**C**H), 127.0 (**C**H), 130.8 (**C**), 132.0 (**C**), 133.3 (**C**H), 135.4 (**C**H), 162.4 (**C**), 180.0 (**C**=O), 180.6 (**C**=O), 189.7 (**C**=O).

4-Keto-β-lapachone **60**: red orange syrup, 5mg (2%); IR: 1767cm^{-1} (C=O), 1712cm^{-1} (C=O), 1698cm^{-1} (C=O); ms: m/z (%), 256 (M^+, 25.7%), 241 (M^+-CH_3, 12.5%), 201 (25.3%), 173 (18.8%), 104 (24.9%), 85 (42.9%), 71 (100%); ^{1}H NMR δ 1.6 (6H,s,C**H**$_3$), 2.7 (2H,s,C**H**$_2$), 7.76 (2H,m,Ar**H**), 8.0 (1H,d,Ar**H**), 8.2 (1H,d,Ar**H**).

2. (0.202g, 0.783mmol) **55** was oxidised by (2.18g, 3.9mmol) CAN with the same procedure as above, but the crude deep yellow product was dissolved in 30mL $CHCl_3$, and then refluxed for 24 hours with aeration. Saturated sodium bicarbonate and brine were used to wash the mixture. Three products were isolated by chromatography on silica gel, using an ether benzene gradient eluant. Compound **57**: 0.114g (57%); Compound **58**: 0.026g (14%); Compound **59**: 0.009g (4%).

VII. BIOTRANSFORMATION OF 4-KETO-α-LAPACHONE 57:

The liquid medium prepared for *Mortierella isabellina* ATCC 42613 1.6L was distributed over 8 1L flasks. A total amount of 0.413g, (1.6mmol) compound **57** was incubated as described above for 72 hours. The crude product was isolated by chromatography on silica gel eluting with a ether benzene gradient, to give 201mg (49%) of 4-hydroxy-α-lapachone **20** as a yellow solid: Rf=0.43 (ether); m.p. 67°C (Lit.:[53] 117-119°C); e.e. > 98% (by chiral shift reagent **8** ^{1}H NMR analysis); $[\alpha]_{589}$= +39.75, $[\alpha]_{546}$= +63.4 (c=0.133,

95% EtOH); IR: 3505cm^{-1} (OH), 1683cm^{-1} (C=O), 1645cm^{-1} (C=O); UV: 250, 280 and 332 (log ε 4.29, 4.13 and 3.44); Mass: found 258.0888, calc. for $C_{15}H_{14}O_4$ 258.0892; ms: m/z (%), 258 (M^+, 23.6%), 243 (M^+-CH_3, 9.7%), 203 (93.4%), 176 (69.4%), 146 (87.3%), 105 (49.5%), 83 (100%); ^{1}H NMR δ 1.44 (3H,s,C**H**$_3$), 1.55 (3H,s,C**H**$_3$), 2.08 (2H,dd,C**H**$_2$-CHOH), 3.80 (1H,br,O**H**), 4.97 (1H,t,C**H**OH), 7.70 (2H,m,Ar**H**), 8.08 (2H,m,Ar**H**); ^{13}C NMR δ 26.76 (**C**H$_3$), 27.1 (**C**H$_3$), 39.6 (**C**H$_2$), 60.0 (**C**HOH); 79.8 (**C**), 120.4 (**C**), 126.0 (**C**H), 126.6 (**C**H), 131.2 (**C**), 131.9 (**C**), 133.4 (**C**H), 134.3 (**C**H), 154.1 (**C**), 180.1 (**C**=O), 186.1 (**C**=O).

VIII. PREPARATION OF 4-ACETOXY-α-LAPACHONE 22:

Acetic anhydride (freshly distilled) (1mL) was added to a solution of 4-hydroxy-α-lapachone (0.071g, 0.27mmol) in 2mL pyridine (dried by distillation from NaOH). The mixture was allowed to stand at room temperature overnight in a stoppered flask. The mixture was then poured into 20mL water, extracted with $CHCl_3$, and the extract dried with $MgSO_4$. After removing the solvent, the crude yellow product was purified by chromatography on silica gel, eluting with an ether benzene gradient to yield 0.080g (97%) **15**. M.p.=145-148.5°C (From EtOH) (Lit: 118-120,[49] 136-140.5°C,[50,53,54]); Rf= 0.576 (ether); $[\alpha]_{589}$=-22.7° ($[\alpha]_{589}$=-14.2°, MeOH)[50], $[\alpha]_{633}$=-21.3° (c=0.0132,MeOH); IR cm^{-1}: 2979 cm^{-1}, 2931 cm^{-1}, 1739 cm^{-1} (OC=O), 1683 cm^{-1} (C=O), 1651 cm^{-1} (C=O) and 1616 cm^{-1}; UV: 244.7 log ε 4.33, 250.4 log ε 4.36,, 280.9 log ε 4.17 and 333.0 log ε 3.52 (245 log ε 4.85, 251 log ε 4.87, 284 log ε 4.71, and 336 log ε 3.99;[50] 245 log ε 4.60, 250 log ε 4.62, 281 log ε 4.47, and 331 log ε 3.70;[53]); Mass for

$C_{17}H_{16}O_5$: found 300.1004, calc. 300.0998; ms: m/z (%), 300 (M^+, 0.6%), 258 (100%), 243 (11.6%), 225 (9.3%), 201 (70.5%); ^1H NMR δ 1.51 (3H,s,C**H**$_3$), 1.56 (3H,s,C**H**$_3$), 2.15 (5H,m,C**H**$_3$+C**H**$_2$), 6.10 (1H,dd,CH_2-C**H**OAc), 7.72 (2H,m,Ar**H**), 8.10 (2H,m,Ar**H**) (Lit.: see Table-6) ; ^{13}C NMR δ 21.0 (**C**H_3), 25.4 (**C**H_3), 28.8 (**C**H_3), 38.1 (**C**H_2), 60.6 (**C**HOH); 78.7 (O**C**), 116.7 (**C**), 126.3 (**C**H), 126.5 (**C**H), 131.1 (**C**), 132.0 (**C**), 133.2 (**C**H), 134.4 (**C**H), 155.8 (**C**), 170.0 (**C**=O), 179.9 (**C**=O), 182.7 (**C**=O).

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